Design and Synthesis of Chemical Probes for the BRPF Bromodomains

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Plagiarism statement

This thesis describes research conducted in the UCL School of Pharmacy, between 24th of September 2012 and 1st August 2016 under the supervision of Professor Paul Fish. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signed…….. **Date**……..

Abstract

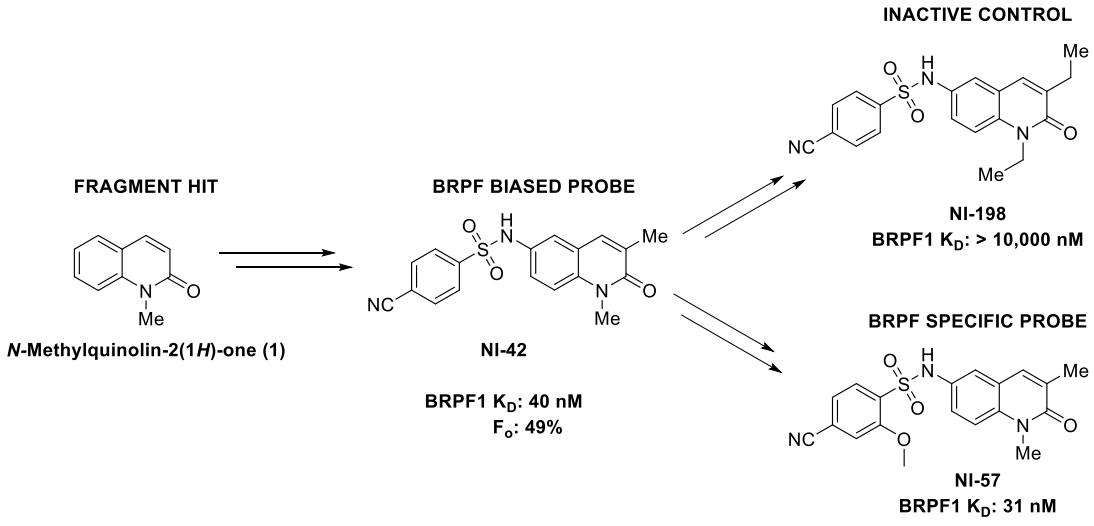
Bromodomains (BRDs) are protein-protein interaction modules responsible for recognition of and binding to ε-*N*-acetylated histone lysines. Following on from the success in drugging the Bromodomain and extra-terminal (BET) domain BRDs, there has been significant interest in elucidating the biological function of the other ~ 50 BRDs encoded for in the human genome.

The BRPF (**Br**omodomain and **P**lant homeodomain **f**inger containing) proteins function endogenously as part of a tetramer involved in regulation of gene transcription, by modulating MYST histone acetyl transferase activity. Translocations and aberrant activity of this tetramer have been implicated in a number of aggressive forms of acute myeloid leukemia (AML), however the role the bromodomain plays in the disease progression is currently unclear.

To this end, BRPF inhibitors were designed by optimisation of the *N*-methylquinolin-2(1*H*)-one (**1**) fragment hit. A credible, tunable SAR model for the BRPF bromodomains, built on the *N*-methylquinolin-2(1*H*)-one core, was developed which has culminated in the synthesis of **NI-42** and **NI-57**, BRPF biased and BRPF specific probes respectively.

Having confirmed the potency and selectivity of **NI-42** and **NI-57**, their pharmacokinetic (PK) profiles were thoroughly investigated highlighting excellent oral and IV PK profiles. Subsequently, the compounds were employed to interrogate the biological consequences of BRPF bromodomain inhibition in a variety of disease models, with some evidence of selective AML cell line growth inhibition being observed.

NI-42 will be of most use when used in conjunction with its inactive control **NI-198**, providing confidence in biological results obtained.



Inhibition of BRPF BRDs by NI-42 shows modest selectivity for inhibiting the growth of AML cell lines

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First and foremost, I wish to thank Professor Paul Fish for giving me the opportunity to work in his lab, for providing advice and for general guidance over the years. Most of all though I want to thank him for being enthusiastic about trying new ideas and always organising new collaborators to evaluate our compounds.

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For chemistry advice, drawing tutorials and even the odd compound I have to thank Elliott, without whom I would still be stuck doing chemistry from the middle ages. I learnt a great deal about chemistry from you, thanks Sensei! Sophie, thanks for answering the odd silly question and perhaps more importantly for putting up with my mess, it can't have been easy.

To my fellow sufferers in G25, we've been through a lot together: explosions (×3!), frequent lab closures, no natural sunlight for 2 years, I could go on … Without you this would have been a whole lot harder. Nik and Mohamed, Waitrose will miss our daily trips for free coffee.

Goeff, thanks for keeping our lab running and dealing with the paperwork over the last year or so. Jess, it's been 5 years now! Thanks again for everything.

Lastly to all my friends and family who would nod and smile while I lamented the latest trials and tribulations of working in the lab, it was much appreciated.

Declaration

This thesis describes research conducted in the UCL School of Pharmacy, between 24th of September 2012 and 1st August 2016. I declare that all the work detailed was carried out by NI with the following exceptions:

All biochemical and biophysical assays were carried out by collaborators. Specifically, thermal shift assays were carried out by Dr Oleg Fedorov at the SGC. In addition, dissocation constants (K_D's) were determined by DiscoverX or the SGC.

All biological and pharmacokinetic experiments were carried out by collaborators as detailed in the text.

All compounds were synthesised by NI with the exception of compounds **189,190** and all the compounds from the large scale synthesis of **NI-57** (Scheme 44, page 110).

Abbreviations

General

AML	acute myeloid leukaemia
app.	apparent
aq.	aqueous
Asn	asparagine
ATAD	ATPase family AAA domain-containing protein
AZ	AstraZeneca
BET	bromodomain and extra terminal
BRD	bromodomain
BRPF	bromodomain and PHD finger containing protein
CETSA	cellular thermal shift assay
CNS	central nervous system
CYP	cytochrome p450 enzyme
DNA	deoxyribonucleic acid
DNMT	DNA methyl transferase
ECT	electroconvulsive therapy
eq.	equivalent
ESI	electron spray ionisation
FRAP	fluorescence recovery after photobleaching
GI_{50}	concentration required for 50% inhibition of maximal cell growth
GSK	GlaxoSmithKline
HAT	histone acetyl transferase
HBO1	human acetylase binding to ORC1
HDAC	histone deacetylase
hEAF6	human ESA associated factor 6 homolog
HRMS	high resolution mass spectrometry
Hz	Hertz
IC_{50}	concentration required to inhibit 50% of the protein
ING (4/5)	inhibitor of growth (4/5)
IR	infrared
ITC	isothermal titration calorimetry
iv	intravenous
J	coupling constant
KAc	acetyl lysine
K_D	dissocation constant
LE	ligand efficiency
LipE	lipophilic efficiency (LipE = pK_d –ClogP)
MOZ	monocytic leukaemic zinc finger
mRNA	messenger RNA
NcoA3	nuclear receptor co-activator 3
ncRNA	non coding ribonucleic acid
NMR	nuclear magnetic resonance
NSI	nanospray ionisation
po	per os (oral)
PCAF	P300 CBP associated factor
PHD	plant homeodomain
Phe	phenylalanine
PK	pharmacokinetic
ppm	parts per million
PTM	post translational modification
qPCR	quantitative PCR
SAM	S-adenosyl methionine
SAR	structure activity relationship
sat.	saturated
Ser	serine
SGC	Structural Genomics Consortium
siRNA	short interference RNA

SUMO	small ubiquitin like modifier
TIF2 (TRIM24)	transcriptional intermediary binding factor 2
TS (T_m)	thermal shift
Tyr	tyrosine
WPF shelf	tryptophan, proline, phenylalanine shelf
δ	chemical shift

Chemical

Me	methyl
AcOH	acetic acid
Boc	*tert*-butoxylcarbonyl
BuLi	butyl lithium
*c*Pr	cyclopropyl
*c*Hex	cyclohexyl
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMA	*N,N*-dimethylacetamide
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
HPLC	high pressure liquid chromatography
*i*Pr	*iso*-propyl
*m*CPBA	*meta*-chloroperbenzoic acid
MW	molecular weight
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
NOESY	nuclear oberhauser effect specroscopy
Ph	phenyl
PPA	polyphosphoric acid
Quant.	quantitative
R	alkyl/aryl subsituent
RT	room temperature
*t*Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THQ	3,4 tetrahydroquinolin-2(1*H*)-one
TLC	thin layer chromatography
TMEDA	tetremethylethylenediamine
μw	microwave

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1. Introduction

1.1 Structure of Chromatin

Deoxyribonucleic acid (DNA), the biological macromolecule that encodes all the information in the human genome, is located in the cell's nucleus and, in order to fit inside the cell, must adopt a condensed highly ordered structure called chromatin. [1]

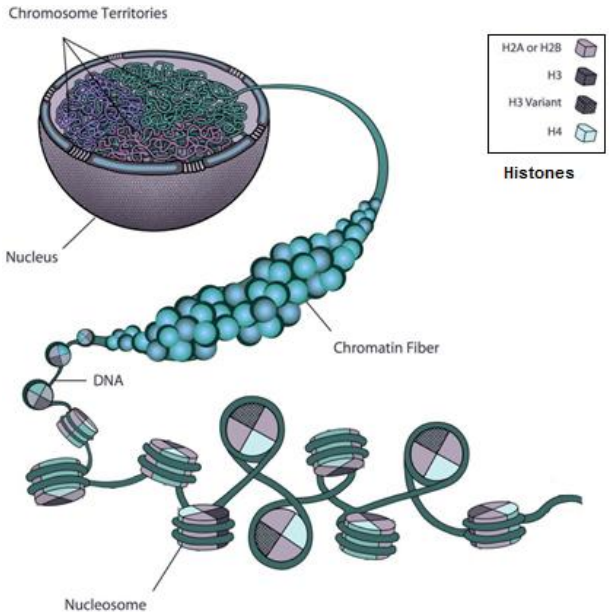


Figure 1. Higher order structure of DNA: DNA is wrapped around histone octamers to form nucleosomes which aggregate to form chromatin. The tightly packed chromatin allows efficient packing of the DNA into the nucleus. Adapted from Shaw et al. [2]

Chromatin consists of negatively charged DNA, positively charged histone proteins and RNA. It is subcomposed of nucleosomes linked by internucleosomal histones.

The nucleosome consists of 146 DNA base pairs wrapped in a double helix around an octamer of histone proteins (Figure 1). The overall structure of the chromatin varies according to the stage of the cell cycle as well as DNA and or histone tail modifications. The importance of the chromatin structure lies in its ability to control gene expression depending on the packing adopted: [1]

1. Euchromatin - Chromatin is tightly packed, this makes it difficult for cellular transcription machinery to access the DNA leading to low transcriptional activity.
2. Heterochromatin – DNA is still wrapped around the histones but no longer adopts the histone octamer, more resembling 'beads on a string'. This more open structure is associated with transcriptionally active genes.

3. During metaphase of cell meiosis chromatin forms higher order structures involving alternative H1 histone analogues for maximum strength.

1.2 Epigenetics

Controlling whether the DNA is packed in a closed form (euchromatin) or more open form (heterochromatin) is vital to the cell's ability to control which genes are expressed. The mechanisms by which this change in gene transcription is controlled, with no concurrent change in gene sequence is called epigenetics.

Whilst research has typically focussed on the genetic basis for disease there is ample evidence that drugging epigenetic processes can provide alternative approaches to challenging diseases like cancer,[2] inflammation, [3] central nervous system (CNS) diseases [4] and others. [5]

Typically epigenetic phenotype changes are brought about by 3 main mechanisms, which are often interlinked (Figure 2): [6]

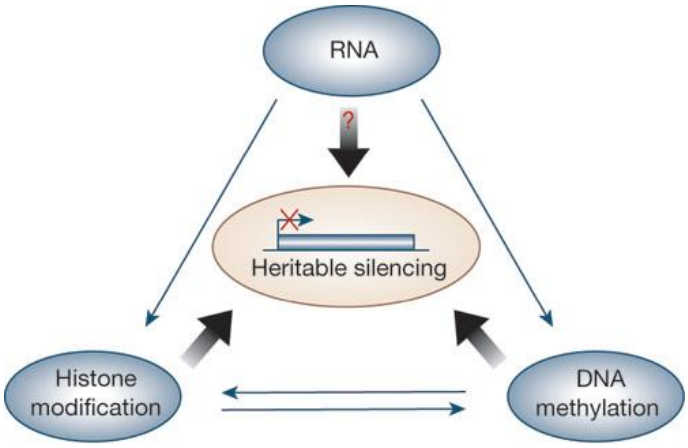


Figure 2. mechanisms for epigenetic phenotypic change showing how they can influence one another. Reproduced from Egger et al. [6]

1. **DNA methylation**– A common method for gene silencing, only cytosine nucleobases located adjacent to a guanine can be methylated (Figure 3). DNA methylation is highly mutagenic, being a frequent cause of C:G to A:T mutations. As a consequence of these CpG islands (> 500 base pairs, > 55% C:G content) are evolutionarily conserved areas of low DNA methylation found in promotor regions of the genome. Indeed, abnormal methylation of CpG islands is typical in many human cancers and is believed to be a key factor in oncogenesis. [6]

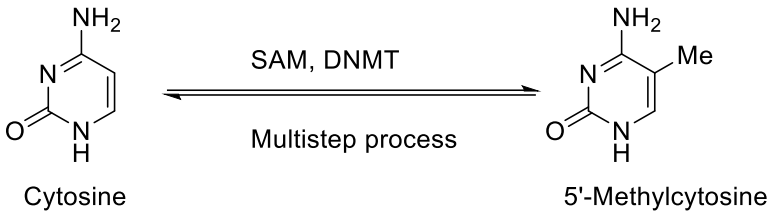


Figure 3. Cytosine methylation: A dynamic process which involves transfer of a methyl from S-adenosyl methionine (SAM) to cytosine catalysed by DNA methyltransferases (DNMTs). DNA demethylation can occur via passive demethylation (concurrent DNA methylation during DNA synthesis) or active (independent of DNA synthesis). In both cases it is a multistep process.

DNA methylation is the epigenetic method for long term information transfer and is responsible for generational (or even transgenerational) epigenetic heritability.

2. **RNA post transcriptional silencing** – This is carried out by a diverse set of non-coding RNAs (ncRNAs) - functional RNA molecules that are not translated into proteins - which act *via* other epigenetic mechanisms to induce phenotypic change (Figure 2).

3. **Histone tail post-translational modification –** Histones, the positively charged proteins around which the negatively charged DNA wraps have N-terminal tails which can be dynamically modified to alter the local structure of DNA. This epigenetic change tends to be much more malleable and transient than DNA methylation, however, histone post translational modifications (PTM) can also lead to DNA methylation and hence long term (transgenerational) genetic changes.

1.3 Histone Tail Post Translational Modifications

Histones are positively charged proteins, divided into 5 families H1/5, H2A, H2B, H3 and H4, whose primary role is providing the support structure for efficient DNA packing. H1/5 histones link nucleosomes, whilst the other 4 families are considered 'core' histones responsible for nucleosome formation.

Nucleosomes are linked by internucleosomal histones which can interact with one another *via* the basic N terminals of their tails. In contrast histones interact with DNA through the basic phosphate backbone of DNA and the positively charged histone tails.

N terminal post translational modification (PTM) of these tails can take many forms and can dramatically alter the charge and shape of the histone tails and hence the higher order structure of the DNA. By affecting the higher order structure of the DNA the series of PTMs, called the 'histone code', can have a significant effect on gene transcription.

1.3.1 Readers, Writers and Erasers

Reading and maintenance of the histone code is carried out by 3 families of proteins: 'readers', 'writers' and 'erasers' which recognise and bind, add, and remove histone PTMs respectively

(Figure 4). There is a unique set of enzymes for each PTM, although some enzymes can catalyse modification of differing levels of the same modification i.e., some class I arginine methyl transferases can catalyse the formation of both Rme1 and Rme2as.[7]

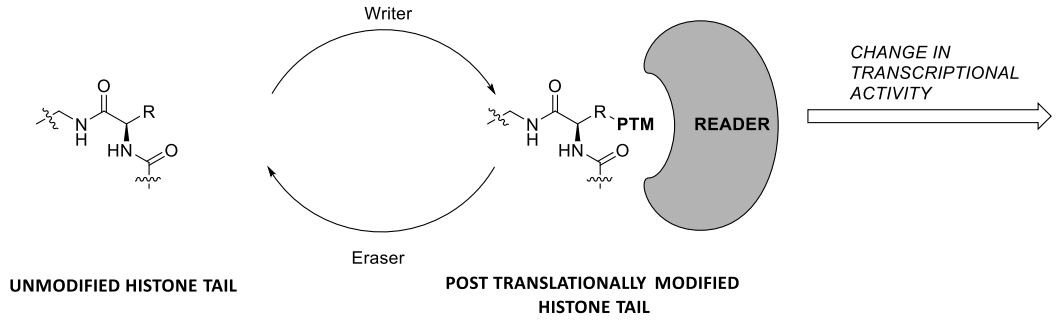


Figure 4. The three protein families responsible for the maintenence of the histone code

1.3.2 Histone PTMs

The first histone PTMs to be catalogued were lysine acetylation and methylation in 1964 by Allfrey *et al.* [8] Subsequently a wide variety of histone PTMs have been discovered, the most common of which are summarized in Figure 5

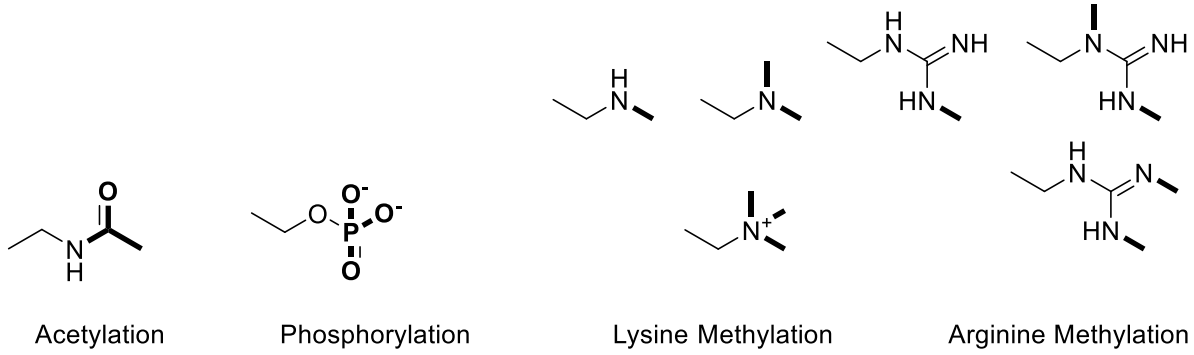


Figure 5. Common histone PTMs with the PTM in bold

Lysine Acetylation – A dynamic PTM that reduces the positive charge of the tail leading to transcriptionally active heterochromatin. [9]

Phosphorylation – can occur on serine, threonine or tyrosine and like lysine acetylation is commonly associated with gene activation.

Lysine / Arginine Methylation - Lysine can be either mono, di or trimethylated whilst arginine can only be mono or dimethylated. However arginine can either be symmetrically dimethylated on the terminal nitrogens or asymetrically dimethylated (Figure 5). Unlike acetylation or phosphorylation, methylation does not necessarily directly affect the charge of the tails. Hence, methylation does not directly affect chromatin spacing. Instead different methylation marks recruit proteins to condense chromatin or cause gene transcription. [10]

1.3.3 Other Histone Post Translational Modifications

A diverse array of less frequent histone tail PTMs have also been catalogued, which either directly affect chromatin structure (like acetylation) or recruit chromatin altering proteins in a similar manner to histone methylation (Figure 6). [9]

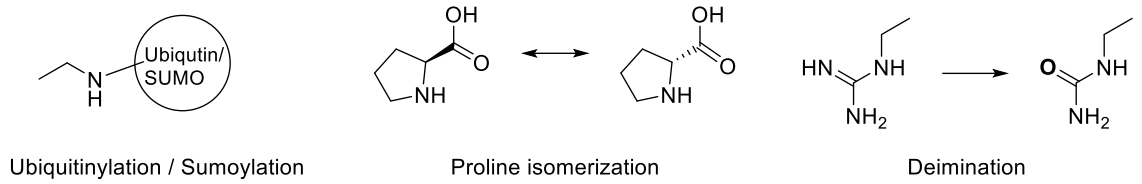


Figure 6. Examples of other Histone PTMs

Ubiquitination and SUMOylation (small ubiquitin-like modifier) are both small proteins usually appended to histone lysines. In contrast to other modifications, the addition of a ~ 70 amino acid protein is relatively large and these modifications can have drastic effects on chromatin structure. Whilst ubiquitinylation can be either repressive or activating, sumoylation is generally repressive. [11, 12]

In contrast, deimination and proline racemization are examples of PTMs that are both amino acid specific and relatively uncommon. There are a number of these specific PTMs whose functions are less well understood. [9]

1.3.4 Histone Acetylation

Acetylation of lysine residues (KAc) on histone tails was one of the first post histone post translational modifications to be characterised, in 1964, by Allfrey *et al* and has long been associated with transcriptionally active areas of chromatin. [8] This is a consequence of the lysine acetylation eliminating the positive charge on the nitrogen and also blocking the interaction of the phosphate backbone with the amine nitrogen (Figure 7)

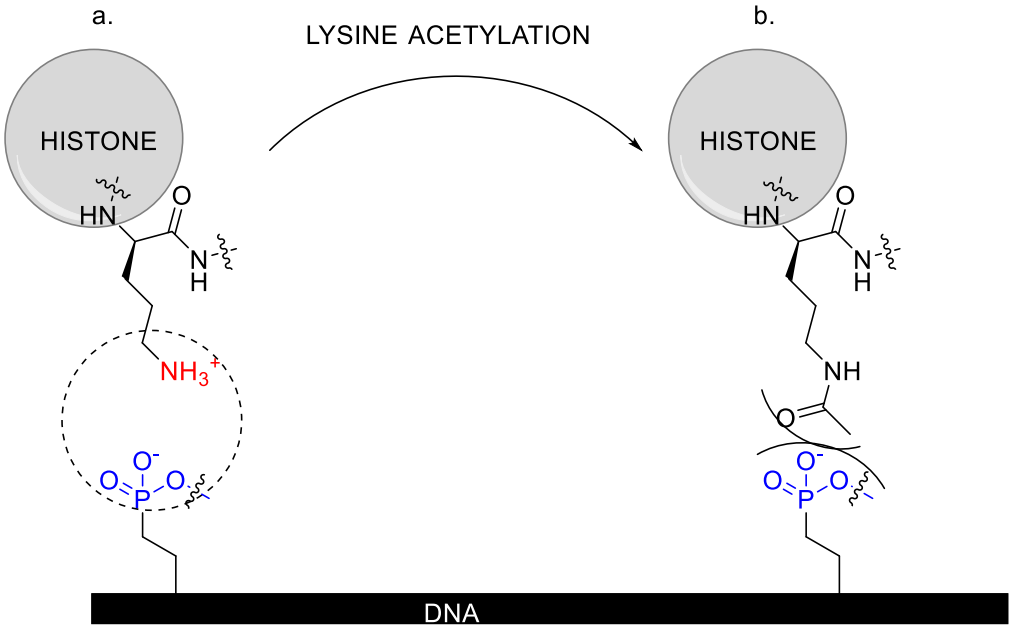


Figure 7. Representation of DNA interacting with histone lysine. a. Positively charged lysine amine can interact with negatively charged phosphate from DNA backbone – condensed euchromatin. b. Acetylation blocks the interaction between the phosphate and lysine and also removes the amine's positive charge – loose heterochromatin

Three families of enzymes are responsible for maintenance of histone KAc levels: Bromodomains (BRDs), Histone Acetyl Transferases (HATs) and Histone Deacetylases (HDACs) which read, write and erase KAc marks respectively (Figure 8). KAc is a highly dynamic PTM and a delicate balance between the enzymes must be maintained, as aberrant acetylation levels have been implicated in a number of different disease states. [13a,13b]

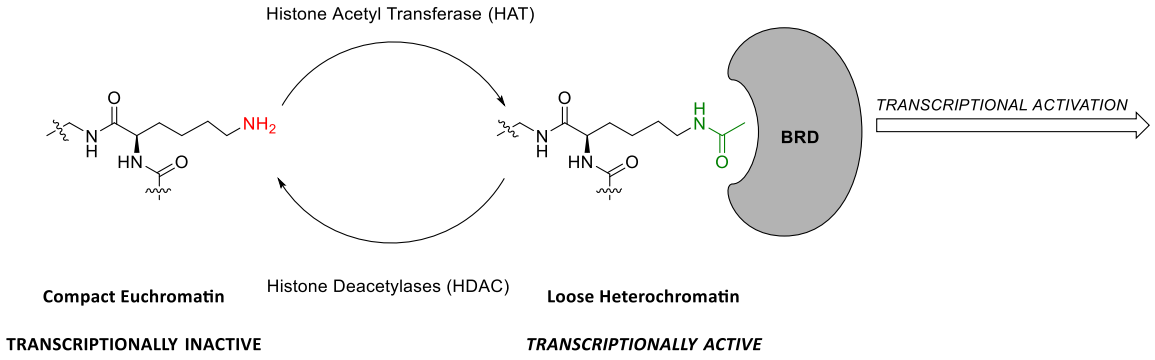


Figure 8. Lysine Acetylation. The lysine ε-nitrogen (red) is acetylated by a HAT (usually with acetyl CoA as a cofactor) to give acetylated lysine (green). Acetylated lysine is bound by a BRD (grey) leading to transcriptional activation. Lysines are deacetylated by HDACs

1.3.4.1 Histone Acetylation State and Disease

HDACs have been linked to several diseases and inhibitors of these enzymes are already in the clinic for the treatment of different cancers (Figure 9). Of interest is the fact that many HDAC inhibitors contain zinc binding motifs (i.e. hydroxamic acids in Vorinostat and Panobinostat) as a consequence of most HDACs having zinc in the binding pocket. [14]

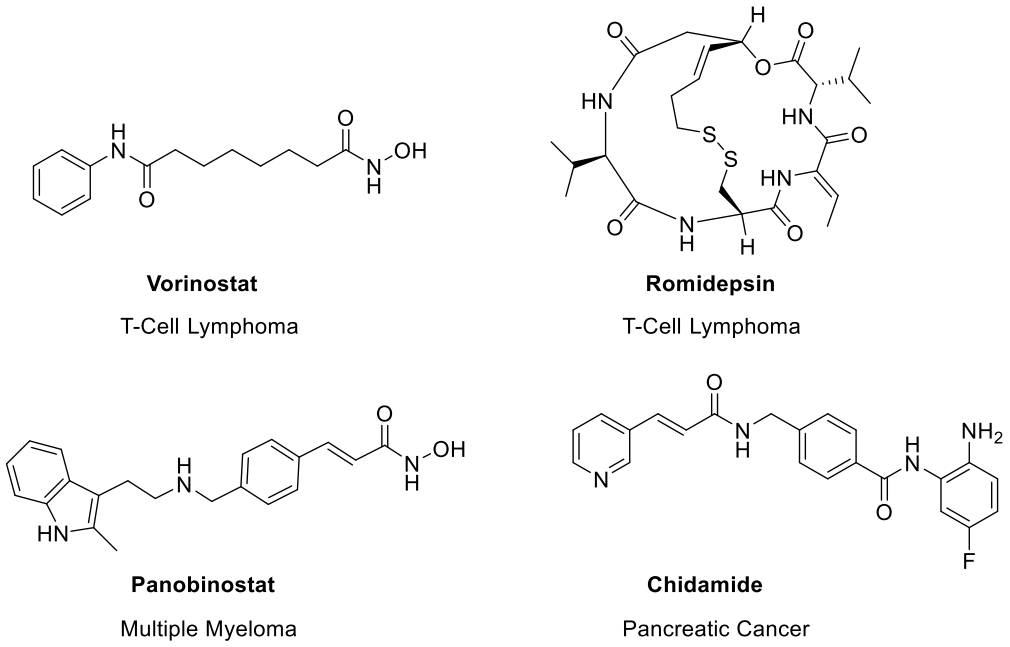


Figure 9. HDAC inhibitors in the clinic with indications

The field of BRD inhibitors is, in comparison, fairly new with the first potent inhibitors only emerging in 2010 (see below). Four inhibitors are currently in clinical trials, 3 structurally similar compounds for haematological malignancies and RVX-208 for atherosclerosis and cardiovascular disease (Figure 10). RVX-208's differing disease indications can be explained by its selectivity for BRD4's second BRD (BRD4 has 2 BRDs). [15]

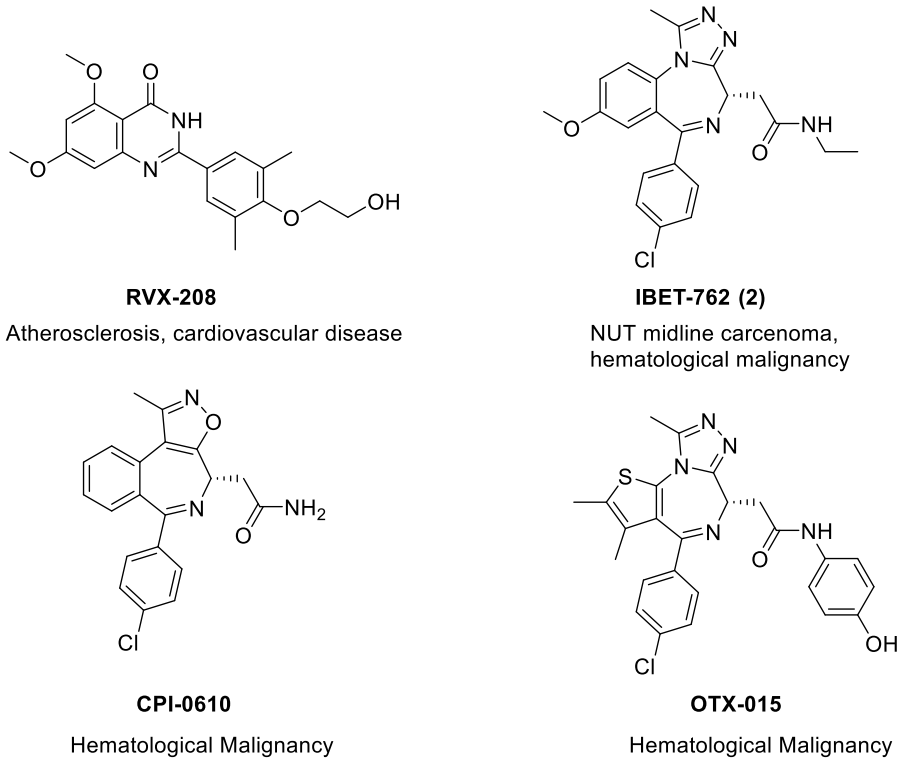


Figure 10. BRD inhibitors with their indications in green

Many small molecule inhibitors of HATs have been synthesised but efforts to evaluate these compounds *in vitro* and *in vivo* have been hampered by very poor cell permeability and stability. Currently only two HAT inhibitors have been evaluated *in vivo*. [16]

1.4 Bromodomains – Acetyl Lysine Readers

Bromodomains (BRDs) were first characterized in 1992 by Kennison *et al* as part of the *brahma (brm)* gene, hence the name, in Drosophila. [17] Several years later (1999-2000) several groups independently elucidated the BRDs biological role as the recognition of ε-*N*-acetylated lysines on histone tails. [18,19]

Later large scale genome analysis revealed that the human genome encodes for 61 BRDs whose biological function is often as part of multiprotein complexes which can contain a wide variety of other epigenetic proteins including: HATs, [20] Lysine Demethylases, PHD domains [21] and many others.

Large scale structural analysis of the 61 human BRDs has shown that they can be sorted into 8 families by sequence alignment (Figure 11). [22]

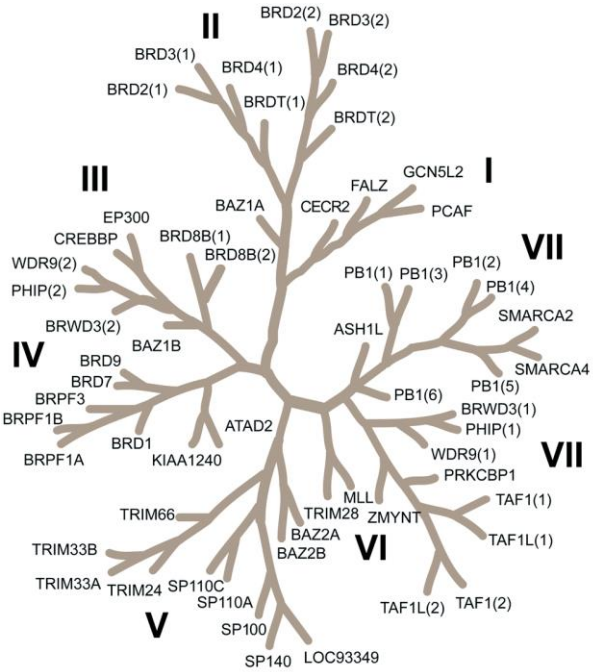


Figure 11. Bromodomain phylogenetic tree showing BRDs sorted into 8 families by sequence alignment

1.4.1 General Structure

BRDs consist of ~110 amino acids organized into 4 left handed antiparallel α helices (α_Z, α_A, α_B and

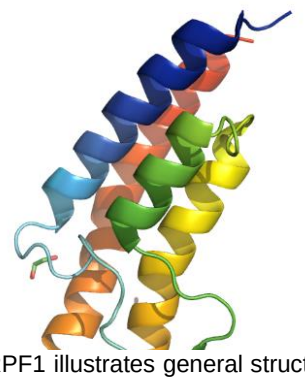


Figure 12. Structure of BRPF1 illustrates general structure of a BRD. αZ = green, αA = Blue, αB= yellow, αC = Red. (PDB 4QYD)

α_C) joined by flexible loop regions (ZA and BC loops) which line the KAc binding pocket (Figure 12). It is variation within these loop regions that affords the BRD's selective binding for different acetylated lysines or small molecules.

Whilst variation is high in the hydrophobic loop regions, the KAc binding pocket is highly conserved with key hydrogen bonds being formed with asparagine and a water mediated hydrogen bond to a conserved tyrosine. Interestingly the water molecules, despite varying in number from 4-6 are crystallographically conserved and very difficult to displace with small molecule inhibitors (Figure 13).

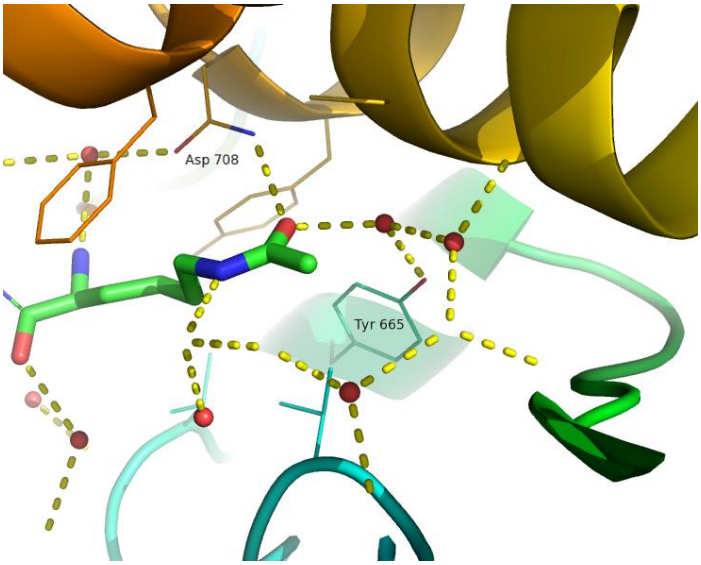


Figure 13. H4K12Ac bound to BRPF1 Conserved Asp 708 and water mediated H-Bond with Try 665 shown (PDB 4QYD).

1.4.2 BRD4 and the BETs

Whilst the existence and biological functions of BRDs have been known for a number of years their roles in disease have remained elusive. In 2006 researchers from Mitsubishi Tanabe identified BRD4 as the primary target from an inflammation phenotypic screen of thienodiazepines.. [23] Further work has associated bromodomains with a range of other diseases including cancers [24] and neurodegenerative disease. [25]

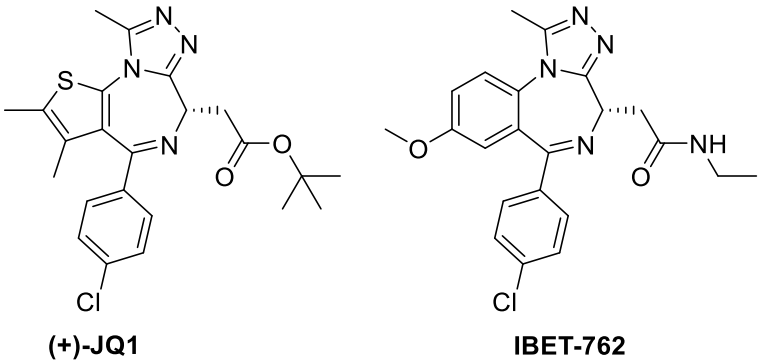


Figure 14. Structure of (+)-JQ1 and IBET-762. Structurally similar BET inhibitors

The first small molecule inhibitors of BRDs were discovered by Zeng *et al*. in 2002, [26] but significant interest in BRDs as therapeutic targets emerged in 2010 when GSK and the Structural Genomics Consortium (SGC) independently discovered potent inhibitors inhibitors of BRD4 (Figure 11, member of the class I BRDs) IBET-762, [27] and (+)-JQ1. [28] respectively (Figure 14).

In large part due to the success of these inhibitors in disease models, and later clinical trials a wave of bromodomain and extra terminal domain (BET) inhibitors has since emerged. These inhibitors have a variety of chemotypes, acetyl lysine binding mimetics and selectivity profiles and have been vital to the scientific communities ability to dissect the biological role of BRD4 and the BETs which have been numerous and far reaching (Figure 10). [29, 30]

1.4.3 Class IV Bromodomains

Following on from the success of BRD4 inhibitors screening campaigns against the other BRD families were initiated in the hope of finding selective inhibitors of non-BET BRDs. This would allow target validation akin to that of BRD4. Of particular interest was the Class IV BRDs as they had been implicated in a number of aggressive diseases with poor prognoses (Figure 15).

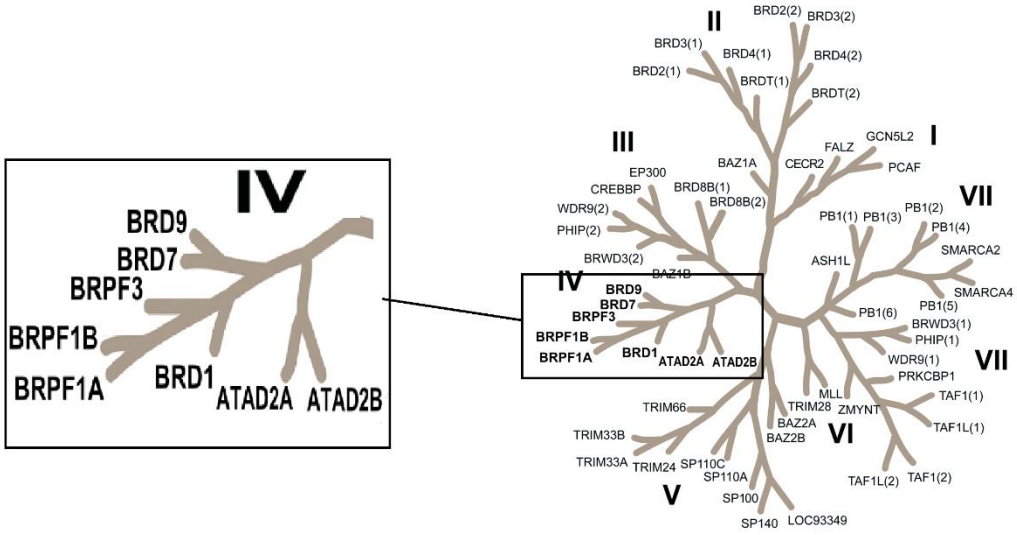


Figure 15. BRD Phylogenetic Tree with Class IV BRDs in bold. NB ATAD2B sometimes referred to as KIAA 1240 and BRD1 sometimes referred to as BRPF2

1.4.3.1 BRD7/9

BRD9 has been reported to be part of SWI/SNF BAF type complexes, mutations of which have been linked with as many as 19.7% of all human tumors. [31, 32] In addition, mutations of BRD9 specifically have been linked with cervical cancer. [33]

BRD7 is also part of SWI/SNF complexes although it is currently believed to be PBAF specific subunit, as opposed to BAF for BRD9. BRD7 has also been suggested as a tumour suppressor gene, as it allows nuclear translocation of p85α causing a decrease in cellular phosphoinositide 3-kinase activity, impairing cell and potentially tumour growth. [34]

1.4.3.2 ATAD/ATAD2B

AAA domain-containing protein 2 (ATAD2) is a protein that contains both an AAA+ ATPase domain and a bromodomain binding region. Misregulation of its activity has been linked to prostate, lung, and triple-negative breast cancers, although it is unknown which domain is responsible for these effects. [35a,35b] The strong cancer disease association correlates well with ATAD2's biological functions as a coactivator for transcription factors which are responsible for genes involved in promoting cell proliferation and survival. [36] Lastly siRNA induced downregulation of ATAD expression has been shown to promote cellular apoptosis. [35a]

1.4.4 BRPFs

The **Br**omodomain and **P**HD **F**inger (BRPF) containing proteins BRPF1, BRPF2 (henceforth BRD1) and BRPF3 are a set of bromodomain containing protein in class IV with >65% sequence identity. [37] They form similar complexes *in vivo,* with the exception that BRPF1 can form a complex with HAT's monocytic leukemic zinc finger (MOZ/MORF) and HBO1 (human acetylase binding to ORC1), whilst BRD1 and BRPF3 can only form complexes with HBO1. In addition BRPF1 has a small N-terminal extension of unknown biological effect (Figure 16). [21] BRPF1 was the first of these BRDs to be isolated in 1994, [38] but the role the trio play in disease and their potential duplication of function and redundancy is only now slowly starting to emerge.

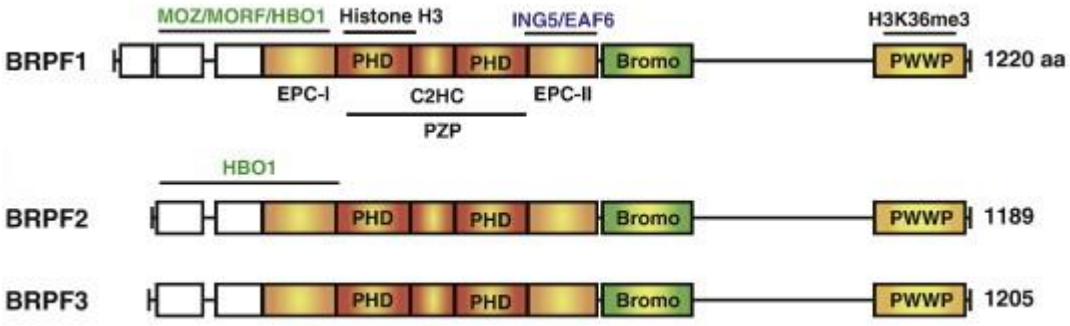


Figure 16. Domain sequence and binding regions for other complex subunits for BRPF1, 2 and 3. Reproduced from Yang *et al.* [21]

1.4.4.1 BRPF3

Genome wide association studies (GWAS) have linked BRPF3 mutations to a number of different diseases. These include the l-citrullin-NO pathway during rodent reproduction, [39] cholangiocarcinomas (bile duct cancer) which account for 10-20% hepatobiliary cancers [40] and synonymous mutation in the *trd1* gene locus involved in regulatory T-Cell development in nonobese diabetic mice. [41] In addition, specific BRPF3 mutations have also been linked to aggressive forms of leukaemia (mean survival time 12 months - adult leukaemia through E419K mutation and paediatric leukaemia through T1031M mutation). [21]

1.4.4.2 BRPF2 (BRD1)

Many of the disease states associated with BRD1 can be linked with its control of H3K14Ac levels. These are controlled by BRD1 bridging and joining HBO1 with its activator protein ING4 (inhibitor

of growth 4). [42] If either HBO1 or BRD1 is depleted in mice knockout studies H3K14Ac levels are significantly lowered. Similar depletion of ING4 lowers the H3K14 levels but the effect is not as dramatic.

One effect of lowering H3K14Ac levels is the knockdown of fetal erythropoiesis. The ensuing anaemia is thought to be the cause of the unviability of BRD1 knockdown mice. [42-43] Unlike BRD1, BRPF3 is not required for survival in fetal mice. [42]

H3K14Ac levels have also been linked to Cd8 (required for killer T-Cell differentiation) expression in the thymus. Insufficient acetylation, as a result of lower than normal BRD1 levels, has been shown to cause abnormal Cd8 expression in CD4+CD8+ double-positive thymocytes (haematopoietic progenitor cell present in the thymus). Deletion of BRD1 by siRNA knockdown showed correspondingly low levels of CD8 expression. [44]

In addition to being required in erythropoiesis, BRD1 expression levels have been linked with electroconvulsive therapy (ECT) for drug resistant severe depressive disorders. After ECT administration, hippocampal BRD1 messenger RNA (mRNA) levels were quantified and a strong dose dependence was observed between number of ECT treatments and BRD1 mRNA. .BRD1-S (short BRD1 mRNA) showed a positive dose dependence with ECT whilst BRD1-L (long BRD1 mRNA) showed a negative dose dependence. The observable time period from ECT treatment to improved mental status suggests that epigenetic reprogramming may be responsible for improved mental status and might represent a novel drug target for psychiatric disease. [45]

1.4.4.3 BRPF1

BRPF1 is the most characterized member of the BRPF family. BRPF1 exists as 2 splice variants BRPF1A and BRPF1B where the splice variant BRPF1A is incapable of binding KAc because of a 12 amino acid insertion in the ZA loop (unpublished work by the SGC).

BRPF1B's (henceforth BRPF1) biological function has been well characterized in a number of different systems and, similarly to the other BRPF members, it performs a bridging role between MOZ HAT and the other members of its biologically active tetrameric complex:human ESA1 associated factor 6 homolog (hEAF6) and inhibitor of growth 5 (ING5) (Figure 17). Each of these complex subunits has multiple epigenetic domains highlighted in Figure 17.

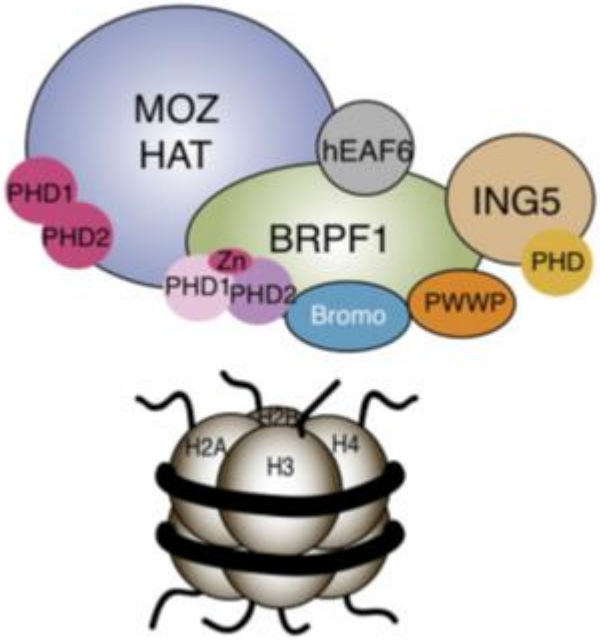


Figure 17. BRPF1 tetrameric complex showing general structure and linking role of BRPF1. Different epigenetic reader domains highlighted reproduced from Poplawski *et al.* [49]

The interplay between the reader domains and their control of the MOZ HAT activity is thought to begin with the ING5 PHD domain binding H3K4me3 and subsequent recognition of unmodified H3 by the MOZ tandem PHD domains. This association of the tetrameric complex to chromatin allows the MOZ HAT to acetylate H3K14,H4K5, H4K8 and H4K16. Lysine acetylation promotes binding of the BRPF1 BRD further strengthens the complex chromatin association, promoting increased HAT activity and creating a positive feedback loop. [46]

1.4.4.3.1 BRPF1 and disease

The MOZ HAT and the complex it forms with BRPF1 was first identified in a chromosomal translocations found in a rare subtype of acute myeloid leukemia (AML) associated with a poor prognosis (median survival - 6 months). This t(8;16)(p11;p13) translocation involved MOZ forming a fusion partner with the CREB binding protein (CBP) HAT in which both HATs retain acetylation activity. Indeed overactivity of these two domains in this translocation have been linked to leukemogenic transformations and oncogenesis. [47]

MOZ/BRPF translocations have also been identified with the CBP homolog p300, the transcriptional intermediary binding factor 2 (TIF2) and the nuclear receptor co-activator 3 (NcoA3) transcription factor. Similarly to the CBP translocation, in the p300 translocation both the HAT and the MOZ acetyltransferase domains remain intact and functional. The resulting aberrant HAT activity is thought to contribute to leukemogenesis and the development of AML.

2. Project Aims

When we initiated our research project (Sept. 2012) there was a clear disease association between the BRPF proteins and disease. However, there were no small molecule inhibitors of the BRPF bromodomains available to the scientific community to ascertain the role that the BRPF bromodomains played in this association.

Thus, it was the aim of this project to:

1. Develop a fit-for-purpose (potent, selective and cell active) ***chemical probe*** for BRPF bromodomains. This would allow for the bromodomain target validation and provide potential starting points for a future drug discovery program.
2. Develop the probe by optimisation of the ***N*-methylquinolin-2(1*H*)-one (1)** core. This template was chosen as it displayed excellent chemical stability, synthetic tractability and evidence of BRD inhibition.

This was carried out in collaboration with the Structural Genomics Consortium (SGC).

2.1 *N*-methylquinolin-2(1*H*)-one as a BRD inhibitor

N-methylquinolin-2(1*H*)-one (**1**) was first identified as a BRD inhibitor as part of a fragment screen carried out by the SGC and Chaikud *et al.* This highlighted that **1** was a 70 mM hit against the PCAF (P300/CBP associated factor) BRD.[48] Structural elaboration, by amination at the 6 position gave **2**, moving activity into the class IV BRDs. Subsequent coupling of the amine to form sulfonamide **3** narrowed down activity to the BRPF subfamily (Figure 18).

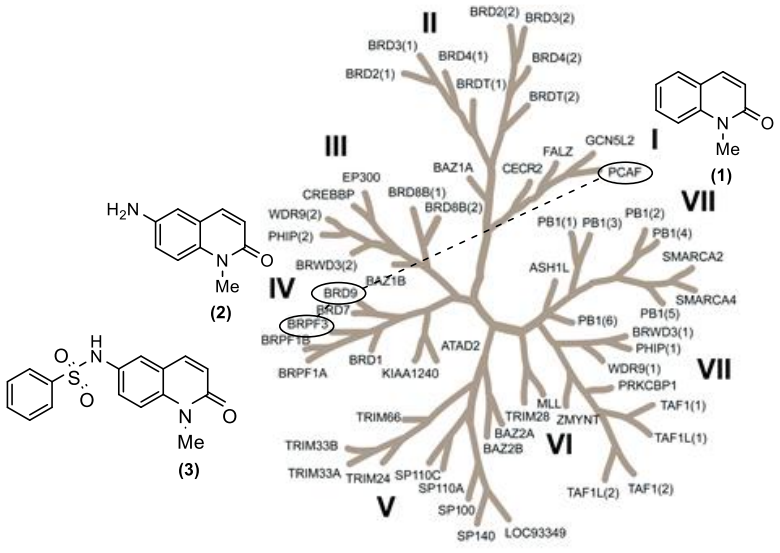


Figure 18. Evolution of fragment hit (**1**) in class I (top right), to amine (**2**) with BRD9 potency (left), to sulfonamide (**3**) with BRPF3 activity (left).

2.2 Criteria for a Fit-for-Purpose Chemical Probe

In order for a chemical probe to provide reliable results a number of generally accepted quality criteria must be met. Our initial goals focussed on meeting the criteria set out by the SGC for their chemical probes: [49]

1. IC_{50} or K_D < 100 nM against primary target
2. 30 fold selectivity over members of the same protein class. 100 fold over proteins of other classes
3. Significant cellular activity at 1 μM

Should these primary goals be achieved, it was envisaged that the more comprehensive criteria set forth by Workman *et al* could then be systematically investigated (Figure 19): [50a, 53b]

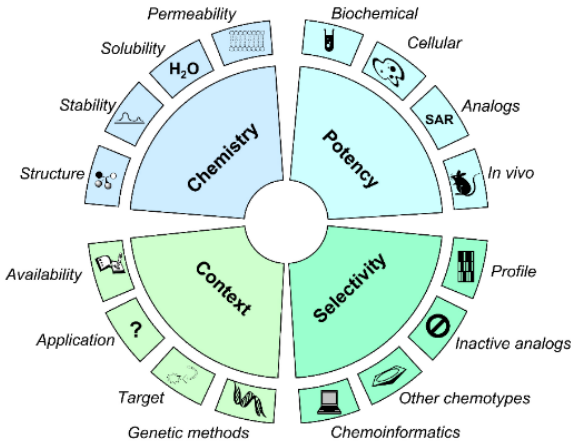


Figure 19. Criteria for a chemical probe, reproduced from Workman et al. [50b]

2.2.1 Selectivity

Profile – Greater than 30-fold selective against all members of the same protein family and greater than 100-fold against all other proteins. Suitable selectivity is important as it imparts confidence that a phenotypic readout from a biological assay can be unambiguously attributed to inhibition of the desired protein.

Inactive control – Determining the biochemical selectivity of a probe is vital to obtaining reliable results, however, it is impossible to screen against all possible intracellular interactions. As such, biochemical selectivity may not truly represent the *in vitro* selectivity. Hence it is desirable to have a molecule that is as structurally similar as possible to the probe, yet inactive. This will help confirm that phenotypic readouts are a result of interrogation of the protein of interest and not a result of off target interactions or assay artefacts.

Structurally orthogonal probe – in a similar vein to having an inactive control, possession of a second probe which is structurally orthogonal in the key binding region of the protein can also validate assay readouts. In the case of BRDs this usually means an alternative KAc mimetic

2.2.2 Chemistry

Aqueous soluble - Compound must be soluble in aqueous media at a sufficient concentration to completely inhibit the protein of interest. Otherwise *in vitro* and *in vivo* application will be limited.

Cell permeable (for intracellular targets) – for cellular *in vitro* models to be reliable, it is necessary for the probe to pass through the cell membrane and interact with the proteins within the cell and or nucleus. Otherwise, a lack of activity in the assay may be misinterpreted as a lack of interaction with the protein of interest.

Structure and Stability – The well characterized chemical structure along with a reproducible synthetic method yielding high purity material, will provide confidence in the material sent for testing. In addition, the stability of the probe in test media is vital.

2.2.3 Potency

Biochemical and cellular potency – For BRDs this has been agreed as < 100 nM biochemical assay potency and < 1 μM intracellular potency, although the values are very target dependent. Greater biochemical than intracellular potency must be achieved to accommodate for the potency decrease usually observed between these two settings. As a corollary to this it is important to measure intracellular concentration as it may vary greatly from assay concentration and may adversely impact results, especially with regard to selectivity windows.

SAR – closely related structures with defined SAR suggest that potency/phenotypical readouts are a result of probe inhibition and not assay noise.

In vivo profile – if the probe is to be used *in vivo* the pharmacokinetic profile must ensure that the intracellular concentration of the probe is high enough to produce a phenotypical result

2.2.4 Context

Genetic corroboration of results – siRNA knockdowns/ knockouts and genetic mutants can provide invaluable comparison to data generated from probe studies.

Availability – Ideally full data on the probe is disclosed with no restrictions on access to material

2.2.5 JQ1 as an Example of a Fit-for-Purpose Chemical Probe

(+)-JQ1 (Figure 20) is a probe developed by the SGC and Jay Bradner for the BET proteins. The BET proteins includes BRD2, BRD3, BRD4 and BRDT, all members of the Class II BRDs (Figure 11). As one of the first bromodomain probes to be discovered **(+)-JQ1** fulfils many of the required criteria for an excellent chemical probe:

1. Potency - K_D – 49 nM against BRD4(1) and 90 nM against BRD4(2).
2. Selectivity - > 30 fold selective against all non-BET BRDs, inactive against 55 ion channels and receptors.
3. Solubility - aqueous soluble.
4. Cell permeability - Localisation to the nucleus and target engagement at < 1 μM confirmed by fluorescence recovery after photobleaching (FRAP) assay.

5. Inactive control – **(-)-JQ1** the enantiomer is inactive against all BRDs and off target proteins.
6. Orthogonal binding motif - **PFI-1** (Figure 20) has very similar selectivity profile with a 1,3-dimethyl-3,4-dihydroquinazolin-2(1*H*)-one core as an alternative KAc mimetic .[51]

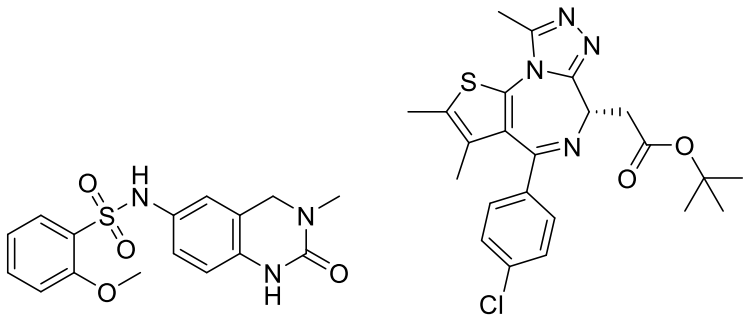


Figure 20. Structures of BRD4 inhibitors **PFI-1** and **(+)-JQ1**

3. Published Inhibitors of the Class-IV BRDs

3.1 Designing an inhibitor of a BRD

The structure of a BRD differs significantly to that of other epigenetic reader domains in that it has a comparatively deep, hydrophobic binding pocket. This makes BRDs ideal targets for the design of small molecule inhibitors. In contrast, chromodomains, which read methylated lysines have charged flat binding pockets consisting of antiparallel strands, making them comparatively difficult to drug.

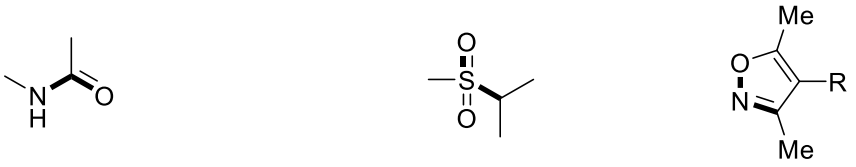
There are a number of key aspects that need to be addressed when designing a BRD inhibitor:

1. The most important interaction in the binding pocket of a BRD is that between the acetylated lysine and the conserved asparagine and tyrosine residues. As such all BRD inhibitors have a warhead that 'mimics' this key interaction. Typically this KAc mimic is itself an amide, but recently more unusual warheads have been disclosed. A selection of KAc mimics is shown in Figure 21.

2. Potency of BRD inhibitors can be increased by designing molecules that interact with the so called 'WPF shelf'. First noticed to be crucial in BRD4 inhibitors this shallow, lipophilic shelf contains repeating units of 3 amino acids, one of which is phenylalanine. Variations of the 2 other amino acids in the shelf can also allow for selectivity in BRD inhibitors. To date only 2 BRD inhibitors have been disclosed which do not intereact with this area of the protein, **PFI-4** (as well as structurally related **GSK5959** and **GSK6853**) and **RVX-208**. [52]

3. BRD binding pockets contain between 4 and 6 conserved deep pocket water molecules which it would be entropically favourable to displace (Figure 13). However, efforts in this regard have proved largely unsuccessful and **PFI-3** is currently the only published inhibitor to be deeply enough bound in the pocket to displace the conserved water molecules. [53]

4. Differences in the 'gatekeeper' residue, an amino acid that lies at the entrance to the KAc binding pocket, can be exploited to confer selectivity of inhibitors. The gatekeeper residue varies in both size and lipophilicity from the small isoleucine in BRD4 to phenylalanine in the BRPFs. Indeed, the residue tends not to vary much between families which can make it difficult to achieve intrafamilial selectivity.



Amides *ie quinolin-2(1H)-ones, most common KAc mimetic iBRD9, PFI-4, PFI-1 etc.*

Sulfoxides. Rare *NVS-CECR2-1*

3,5 dimethylisoxazoles *SGC-CBP30*

Figure 21. Selection of published KAc mimetics (mimetic in bold). Published inhibitors with this mimetic in green

Many of these criteria have been gainfully employed in the design of inhibitors of the class IV bromodomains. An overview of the published inhibitors is presented below.

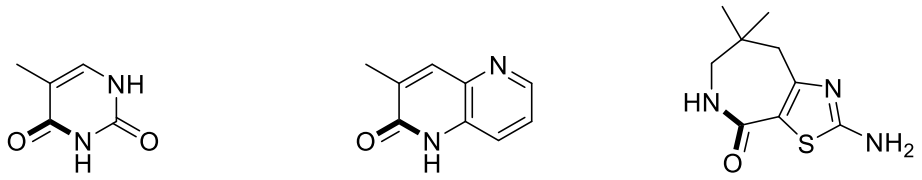
3.2 ATAD2

WPF Shelf Residues: Arginine, Valine, Phenylalanine

Gatekeeper Residue: Isoleucine

With its comparatively shallow and polar binding pocket ATAD2 is considered the most difficult of the class IV BRDs to drug. [54] Despite this, high throughput screening (HTS) of fragments has facilitated the discovery of several potent inhibitors of this BRD.

In 2013 the SGC disclosed fragment hits with modest affinity for ATAD2 based on a uracil template (**4**, Figure 22). [55] Subsequently, in 2014, Harner *et al* disclosed a selection of more potent fragments, although several of the more potent fragments were inversely selective for other members of the class IV bromodomains. [56]



4 **5** **6**

Figure 22. A selection of published fragments that inhibit the ATAD BRD. KAc mimetic in bold

To date, the most potent ATAD2 inhibitors have been disclosed by GSK, with initial publication of **7**, a low μM inhibitor. Subsequent lead optimization of **7** yielded **8**, which was 10 fold more potent an inhibitor of ATAD2. Addition of the pyridine ring at the 5-position maintained both the ligand efficiency (LE **7**: 0.21, LE **8**: 0.20) and the lipophilic ligand efficiency (LLE **7**: 6.34, LLE **8**: 6.34) . Unfortunately, despite maintaining the binding metrics of the lead compound, selectivity of **8** *vs* BRD4, inhibition of which has widespread biological consequences, was insufficient for its use as a probe molecule. [57a, 57b]

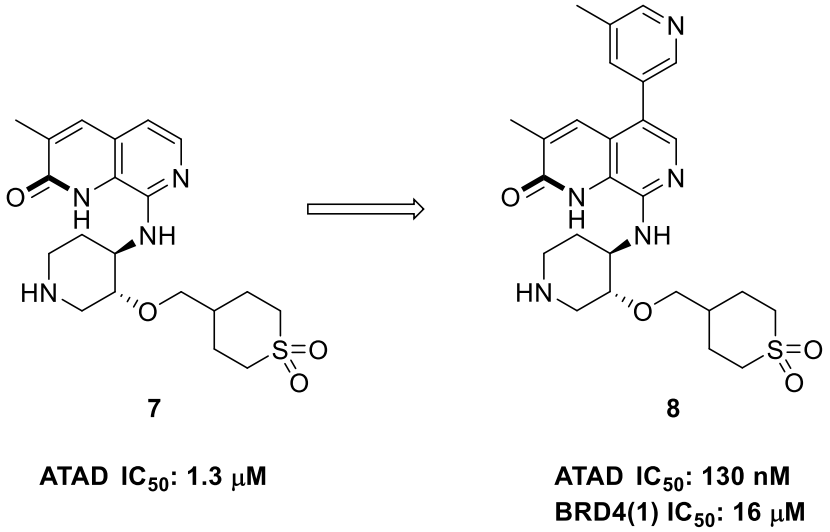


Figure 23. GSK inhibitors of ATAD2 KAc mimetic in bold

3.3 BRD7/9

WPF Shelf Residues Glycine, Phenylalanine, Phenylalanine

Gatekeeper Residue Tyrosine

There has been significant progress in the development of potent and selective inhibitors of BRD9 and BRD7. Whilst most of the reported inhibitors in the literature are dual inhibitors of both BRDs, Theodoulou *et al* have also reported I-BRD9, a BRD9 selective inhibitor (Figure 28). [58]

3.3.1 BRD7/9 dual inihibitors

The SGC and coworkers have published several dual inhibitors of BRD7/9. The first of which, **LP99**, is based on the same core as the work described herein *N*-methylquinolin-2(1*H*)-one (**1**). The core was elaborated by addition of a substituted valerolactam ring at the 7 position and a methyl group at the 4 position of the quinolin-2-one ring (Figure 24).

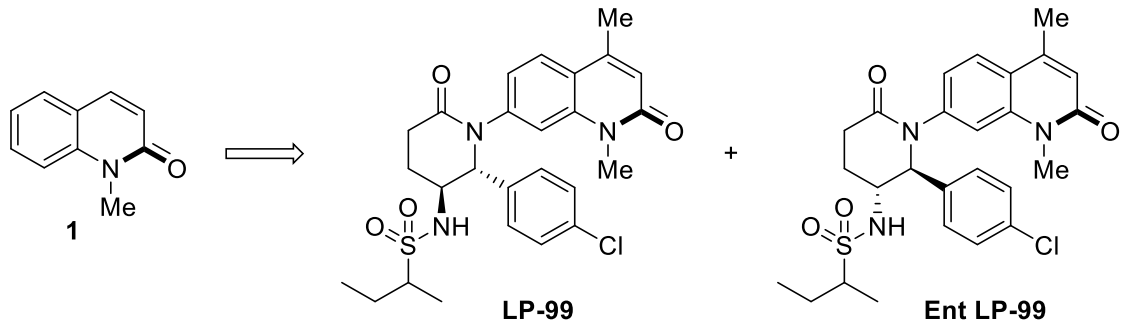


Figure 24. Development of LP99 from *N*-methylquinolin-2(1*H*)-one. KAc mimetic shown in bold

LP99 forms the key binding interactions through the C2 carbonyl in the quinolinone A ring interacting with the conserved Asn 100 and Tyr 57. In addition, the quinolinone A ring forms a beneficial π-stacking interaction with Tyr 106 the gatekeeper residue in BRD9. **LP99** has a potency of 99 nM by ITC K_D, but whilst it is disclosed that there is activity by DSF against BRD7, the potency

of the interaction is not quantified. The enantiomer of LP99 (**Ent LP-99**) is inactive against all BRDs and is an excellent negative control for this inhibitor. [51]

The second SGC BRD7/9 inhibitor was developed from the fragment **9**, which was also used as a starting point towards a BAZ2A/B inhibitor. Substitution of the *n*-propoxy by a morpholine ring and fusion of a pyrrole to the pyridine ring furnished compound **10** (Figure 25).

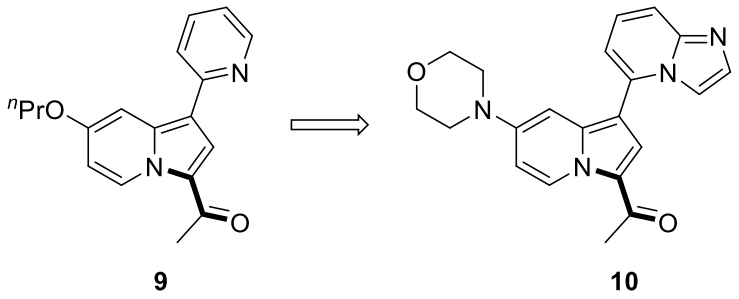


Figure 25. Development of Fragment **7** into BRD7/9 inhibitor **9**

Compound **10** displayed a BRD9 K_D of 68 nM and was selective against all other bromodomains, including BAZ2A/B. Similarly to **LP99** the acetyl was interacting with Asn 100 and Tyr 57, whilst forming a π stacking interaction with Tyr 106. [59]

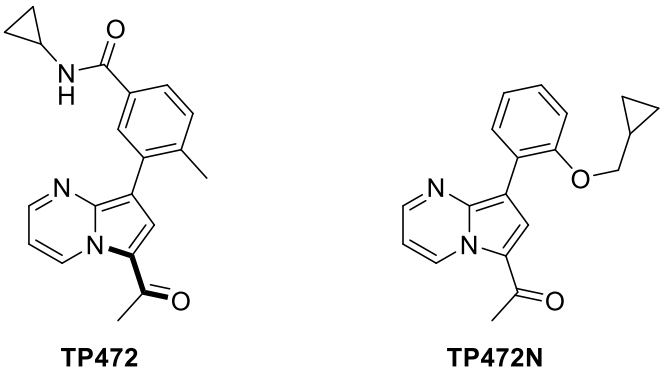
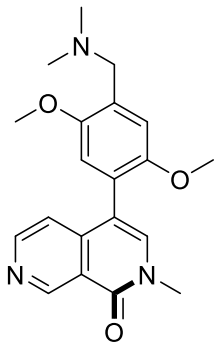


Figure 26. Structure of TP472 and its inactive analogue. KAc mimetic in bold

Recently a number of other BRD7/9 inhibitors have been reported including **TP472**, developed by the SGC in collaboration with Takeda, which is very structurally similar to inhibitor **10.** Also mentioned on the SGC website is negative control compound **TP472N. TP472** is also slightly more potent than **10** with an ITC of 33 nM against BRD9 and 10 fold selectivity over BRD7 for which it has a K_D of 340 nM. [28]

The last of the BRD7/9 inhibitors disclosed at this time is **BI-9564** which is based on a different chemotype to the previously reported inhibitors of the same BRD. **BI-9564** developed in collaboration between the SGC and Böhringer Ingelheim displays potencies of 14 nM and 239 nM against BRD9 and BRD7 respectively. [28]

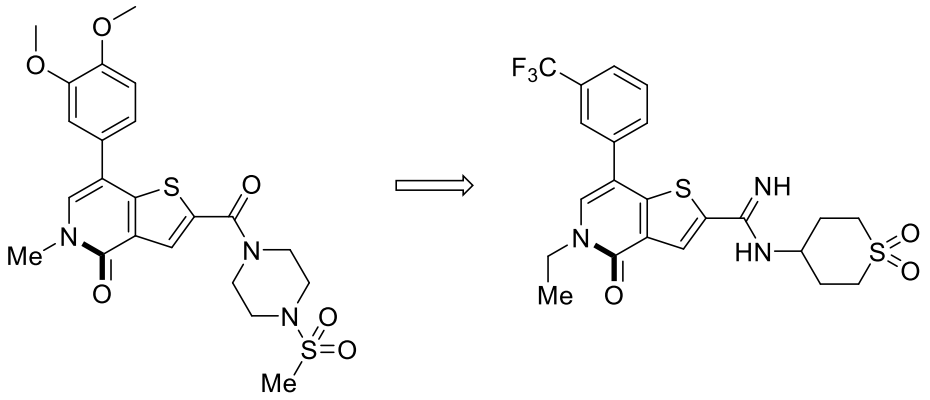


BI-9564

Figure 27 Structure of BI-9564 KAc mimetic in bold

3.3.2 BRD9 Selective Inhibitors

To date, the only BRD9 selective inhibitor is **I-BRD9**, developed by Theodoulou *et al* at GSK. Screening of the internal GSK compound library indentified thienopyridone (**11**) which was 100-fold selective for BRD9 over BRD4. Selectivity was maintained, whilst increasing BRD9 potency by replacement of the carboxamide amidine at the 2 position of the thienopyridone and the introduction of an electron withdrawing 2-trifluoromethylbenzene group to fit into the WPF Shelf in BRD9. [58]



11

BRD9 IC_{50} = 200 nM
BRD4 IC_{50} = 20,000 nM

I-BRD9

BRD9 IC_{50} = 50 nM
BRD4 IC_{50} = 5,000 nM

Figure 28. Optimisation of fragment **11** to give **I-BRD9**

Measurement of BRD7/9 potency by DiscoverX revealed that I-BRD9 was ~ 200 fold selective for BRD9 over BRD7 (BRD9 K_D : 2 nM, BRD7 K_D : 398 nM). The authors suggest that the origin of this selectivity may lie in the replacement of Gly 46 in BRD9 with Ala46 in BRD7, creating a slightly larger pocket for the trifluoromethylbenzene group to occupy.

3.4 BRPF inhibitors

WPF Shelf Residues: Asparagine, Isoleucine, Phenylalanine

Gatekeeper Residue: Phenylalanine

Workers at the SGC, GSK and Pfizer have all reported inhibitors of the BRPF bromodomains based on the 1,3-dimethyl-1,3-dihydro-2*H*-benzo[d]imidazol-2-one core. **PFI-4, GSK5959** and **GSK6853** are all BRPF1 specific inhibitor with large structural similarities, [28, 37, 60] whilst **OF-1** is a pan BRPF inhibitor. [28]

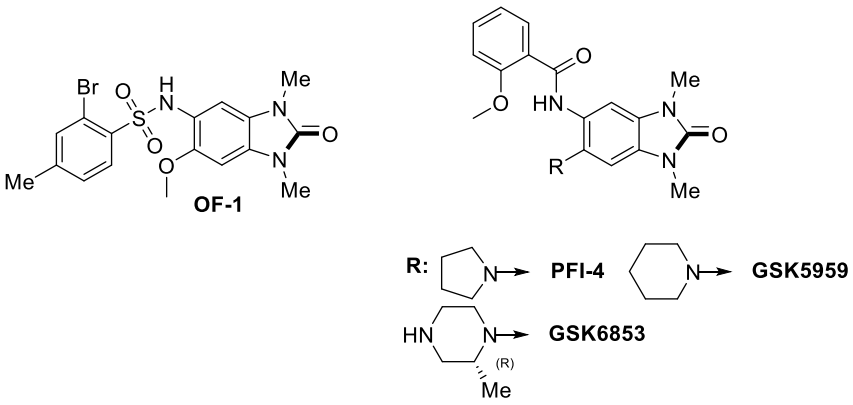


Figure 29. Published inhibitors of the BRPF bromodomains. KAc mimetic in bold.

All of these BRPF1 inhibitors exploit the same key KAc mimetic interactions. The carbonyl bonds with Asn708 and *via* a water mediated hydrogen bond with Tyr665 (Figure 30). Overlaying the *apo*-structure of BRD1 with the cocrystal structure of **GSK6853** suggests that the only difference in the 2 bromodomains that might account for the exquisite selectivity is BRPF1's Pro658 which is replaced in BRD1 by Ser698 and Asn619 in BRPF3. The advantage of the BRPF1 proline lies in its ability to form a stabilising hydrophobic interaction with the 1,3-benzimidazalone ring, an interaction serine and asparagine are unable to form (Figure 30).

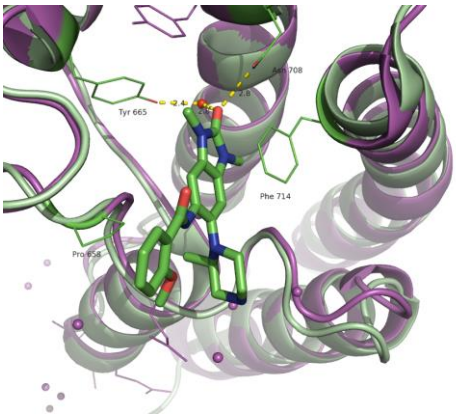


Figure 30. Cocrystal structure of **GSK6853** with BRPF1 (Purple) overlayed with the apostructure of BRD1 (pale green) highlighting the key binding interactions and the hydrophobic interaction between Pro 658. PDB ID: 5G4R

Whilst the 1,3-benzimidazalone core was able to yield a number of potent and selective BRPF inhibitors, the main drawback of this core was its inherent insolubility. It is for this reason that Demont *et al* further developed **GSK5959** into **GSK6853**, derivatising the piperidine ring into a 2-methylpiperazine for increased aqueous solubility.

Demont *et al* have also developed a negative control **GSK9311** for **GSK6853**, which involved *N*-ethylating the amide. It was reasoned that the alkylation would inhibit the formation of the internal

hydrogen bond whilst also promoting a steric clash between the alkyl group and the piperazine ring. Overall these interactions would result in the amide ring being unable to adopt the conformation required to fit into the ZA channel and therefore potency would be reduced. This was in fact the case, as **GSK9311** was 1000-fold less potent against BRPF1. [60]

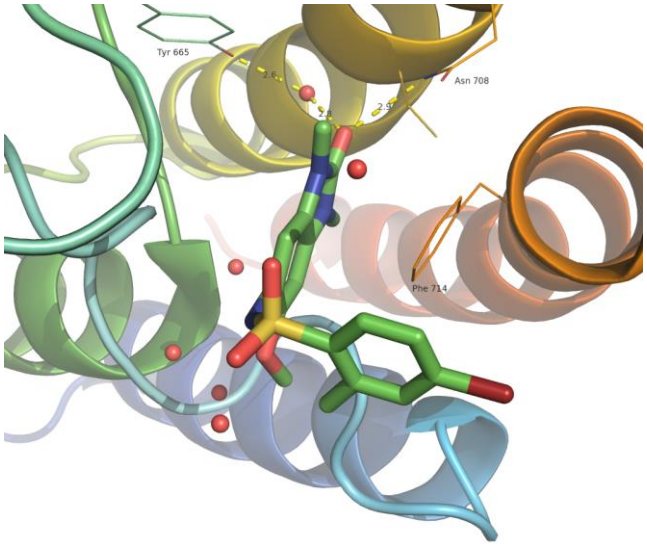


Figure 31. Cocrystal structure of **OF-1** and BRPF1 highlighting the key binding interactions. PDB ID: 5FG4

In contrast to the other BRPF inhibitors **OF-1** is a pan-BRPF inhibitor, recording K_D's of 100, 500 and 2,400 nM against BRPF1, BRD1 and BRPF3 respectively. This is a result of replacing the amide moiety for a sulfonamide, which angles the aryl ring into the WPF shelf of BRPF1. As the sequence homology between the BRPF bromodomain binding sites is extremely high, without the ability to exploit Pro 658, achieving the selectivity of the BRPF1 specific inhibitors is impossible.

3.5 BRPF/TRIM24 Inhibitors

Two further BRPF inhibitors based on the 1,3-benzimidazalone have been published, featuring large aryl ether substituents at the 5 position of the benzimidazalone core. Addition of these ethers causes these inhibitors to retain BRPF1 whilst gaining appreciable TRIM24 potency.

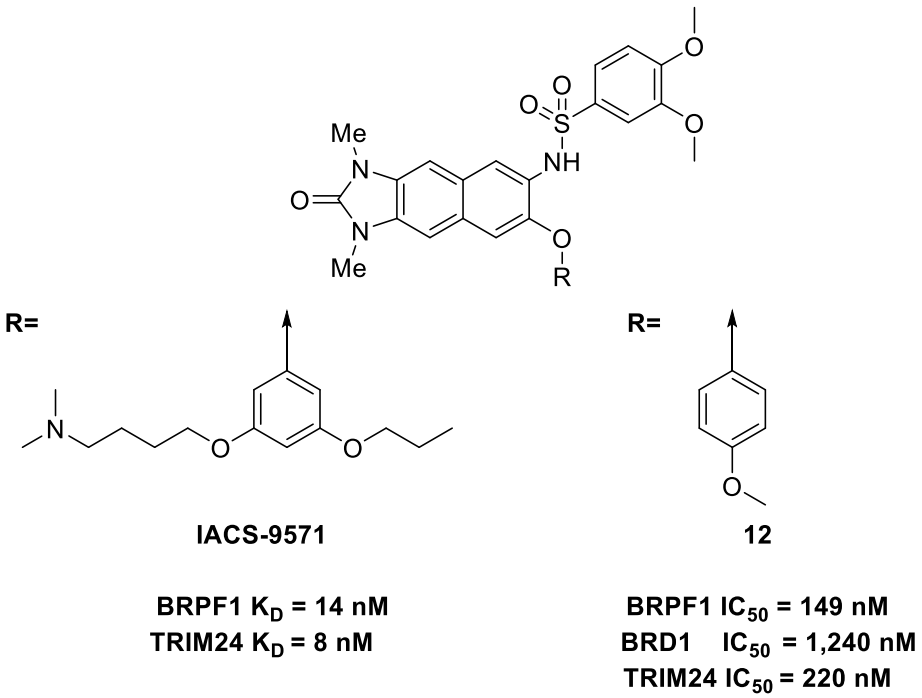


Figure 32. Published dual inhibitors of the TRIM24 and BRPF1 BRDs

Despite its strong potency against BRPF1 **12** was only able to inhibit the TRIM24 BRD-chromatin and not the BRPF1 BRD-chromatin association at 1 μM in an *in vitro* context (FRAP assay). Screening of compound **12** in a cancer cell line panel revealed only modest GI_{50} values (> 10 μM). [61]

In contrast **IACS-9571** displays far greater cellular target engagement, recording an EC_{50} of 50 nM. This may be a function of its improved pharmacokinetic profile allowing greater intracellular concentration. [62]

3.6 BRD1/TAF1 Inhibitors

Recently a collaboration between Bayer and the SGC has disclosed **BAY-299** a dual BRD1-TAF1 BRD inhibitor. **BAY-299** has a K_D of 6 nM against BRD1 and 13 nM against TAF1(2). In addition BAY299N has been synthesised as a negative control. Further details regarding this inhibitor are not currently available. [28]

4. Results and discussion

4.1 Biological Analysis of compounds

Compounds were primarily screened against BRDs of interest by two methods. Initial screening was carried out by Thermal Shift (TS) at the SGC for qualitative confirmation of binding. Subsequent to TS, promising compounds were quantitatively assessed, at DiscoverX, by determining a dissociation constant (K_D).

4.1.1 Thermal Shift

Thermal Shift (TS) measures compound binding to the protein by monitoring the fluorescence change of the dye Sypro Orange as a function of temperature. When the protein is folded the fluorescence signal from the dye is very low, due to quenching of the signal by the aqueous environment; however, on heating and protein unfolding the Sypro Orange binds to hydrophobic regions of the protein and a marked increase in fluorescence can be observed. The fluorescence intensity is then plotted against temperature, from which the melting temperature or T_m can be calculated (Figure 33). The higher ΔT_m the more stable compound:protein complex is, which is indicative of a higher binding affinity.

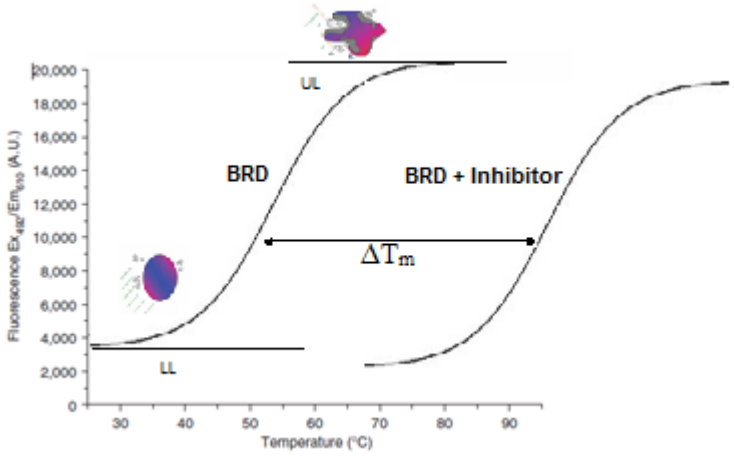


Figure 33. Example of a Thermal Shift plot with fluorescence plotted against temperature. The differences in the melting temperatures (ΔT_m) of the BRD and the BRD with the inhibitor is directly proportional to compound affinity. Adapted from Niesen *et al.* [63]

Despite its excellent characteristics as a high throughput assay (small amounts of protein, fast, operational simplicity and good qualitative ordering of compound binding), TS assays suffer from a number of important drawbacks. These include qualitative results that do not scale from protein to protein, and as T_m is measured by fluorescence, binding of lipophilic compounds to Sypro Orange can artificially increase the measured T_m. Although rare, it is also possible for compounds to inhibit the fluorescence of Sypro Orange.

4.1.2 Dissociation constant (K_D)

Dissociation constants (K_D) are a quantitative measure of compound binding by assessing the position of the equilibrium between the unbound BRD and inhibitor, and the BRD:inhibitor complex (Figure 34).

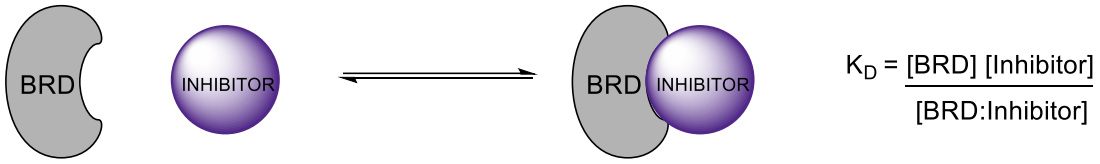


Figure 34. Graphical representation of a K_D. On the left of the equilibrium the BRD (grey) is not bound to the inhibitor (purple). On the right of the equilibrium the BRD and inhibitor have formed a complex. A low K_D indicates that the equilibrium lies to the right.

Dissociation constants were determined by DiscoverX *via* a competition assay with an immobilised ligand of known affinity, followed by quantitative polymerase chain reaction (qPCR) analysis of the tagged BRD still bound to the immobilised ligand.[64] Initially, compounds are incubated with immobilised ligands of known K_D bound to DNA tagged BRDs . Compounds that inhibit the bromodomain prevent its binding to the immobilised ligand, thus reducing the amount of protein captured on the solid support. K_D's are determined by qPCR measurement of the amount of DNA tagged BRD still bound to the known inhibitor and plotting the value obtained versus the concentration of the test compound (Figure 35).

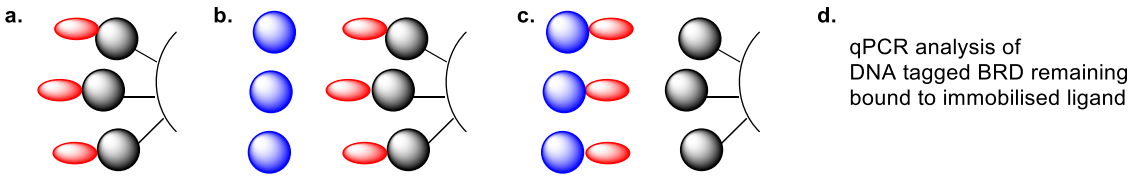


Figure 35. Graphical representation of K_D determination as carried out by DiscoverX. A. DNA tagged BRD (red) is bound to the immobilised ligand (black). b. No competition on introduction of test compound (blue). The test compound is not sufficiently potent to displace the bound BRD from the immobilised ligand. c. The test compound is sufficiently potent to displace the immobilised ligand and is now bound to the DNA tagged BRD. d. qPCR analysis of the the amount of DNA tagged BRD remaining bound to the immobilised ligand allows for determination of a K_D. Adapted from DiscoverX.[65]

The primary advantage of a dissociation constant in comparison to TS is that the quantitative data obtained can be compared from protein to protein. Moreover, as nearly the entire bromonome is available for screening it is now possible to profile compounds against the entire BRD family (protein class) in the same assay format, allowing for meaningful comparison of data.

However, the assay as carried out by DiscoverX has a couple of drawbacks. Chief amongst these is the dependence on having a suitably potent inhibitor as the 'known affinity ligand'. If the test compound is orders of magnitude more potent than the known inhibitor the values derived from the assay can be adversely affected. This is due to the fact that the percentage change in the amount of 'known inhibitor' displaced will be well within the error of the assay beyond a certain point. In addition, as dissociation constant determination is a commercial assay, the cost of obtaining all the relevant data is can be prohibitively high.

4.1.3 Interpretation of Thermal Shift and Dissociation Constant Data

TS values were compared with those previously determined by the SGC for the relevant protein before the values were used for qualitative ranking of compounds. Based on SGC expertise and data BRPF1 TS values were then bracketed as follows:

TS < 2 °C being considered inactive

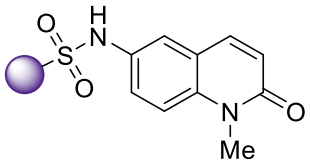
2 °C < TS > 5 °C being considered moderately active

5°C < TS being considered very active

When assessing dissociation constants as determined by DiscoverX a potency difference of ~ 3 fold or greater was considered significant. Ultimately, ITC K_D's as determined by SGC were employed to definitively assess compound selectivity and potency.

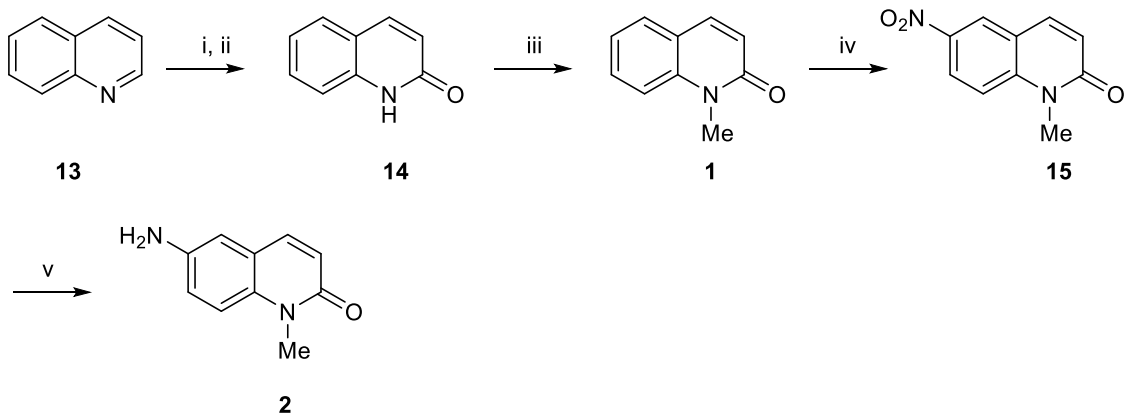
4.2 Lead Compound to BRPF probe – SAR

4.2.1 Lead compounds and Initial SAR



Initial structure activity relationship (SAR) data gathered by the SGC on sulfonamides (unpublished data from the SGC) focussing on simple mono or di substitution of the sulfonamide aryl ring was inconclusive as to position and electronic effect of different substituents.

As such our studies began by synthesising simple alkyl, cycloalkyl and benzenesulfonamides in order to validate the initial results against BRPF1. Our synthesis is outlined in Scheme 1.



Scheme 1. Reagents and Conditions: i) *m*CPBA, CH_2Cl_2 ii) Benzoyl chloride, NaOH (1.0 M), CH_2Cl_2, 78% over 2 steps; iii) NaH, MeI, DMF, 69%; iv) H_2SO_4, HNO_3, 0–5 °C, 87%; v) $SnCl_2$, HCl, 97%.

Oxidation with *m*CPBA in CH_2Cl_2 gave quinoline-*N*-oxide in quantitative yield. Preliminary conditions for addition to the *N*-oxide, followed by rearrangement, focussed on tosyl chloride, K_2CO_3 in $CHCl_3$, however reproducing literature conditions afforded **14** in low and variable yield

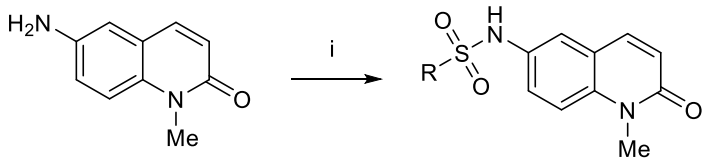
(25 – 51%). [66] Rearrangement with acetic anhydride in acetic acid proved similarly unsuccessful, however conditions employing benzoyl chloride and aqueous sodium hydroxide proved reproducible and high yielding (78 -84 %, n = 3).

With reproducible, scalable conditions in hand for the rearrangement to quinolin-*2*(1*H*)one (**14),** methylation was either carried out using K_2CO_3 (small scale ~ 100 mg) or NaH (large scale ~2 g) to afford *N*-methylquinolin-*2*(1*H*)one (**1**) in moderate yields.

Subsequent nitration by dropwise addition of HNO_3 (68%) to a solution of **1** in conc. H_2SO_4 at 0 °C yielded the desired six position nitro isomer (**15**) as confirmed by ^{1}H NMR (doublet at 8.75 ppm for C_5H and a doublet of doublets at 8.41 ppm for C_7H) and the literature. [67] Nitro group reduction of **15** with $SnCl_2$ (either anhydrous or dihydrate) gave amine **2** in good yield.

4.2.1.1 Simple Alkyl Sulfonamides

Amine **2** was then routinely coupled in moderate to good yields with simple alkyl, cycloalkyl or benzenesulfonyl chlorides in DCM with pyridine (Scheme 2) to afford **3** and **16**-**20** for testing of inhibition of BRPF1 (Table 1).



1 **3, 16-20**

Scheme 2. Reagents and Conditions: i) RSO_2Cl, Pyridine, DCM.

Compound	R Group	Yield	ΔT_m against BRPF1 (°C)[a]	K_D against BRPF1 (nM)[b]	ClogP
2	-	NA	n.d	2100	0.45
16	Me	59	1.4	2200	0.37
17	Et	64	1.8	1200	0.90
18	iPr	41	2.2	1100	1.21
19	cPr	97	1.9	520	0.96
20	cHex	58	3.9	380	2.40
3	Ph	47	5.1	29	2.00

Table 1. Biochemical and biophysical evaluation of compounds **3,16-20**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top

concentration. Data is the mean of 2 replicates. ClogP's calculated by ChemBioDraw Ultra 14.0 throughout thesis

Pleasingly, a trend in BRPF1 affinity can be observed, where increasing the length of the carbon chain and increasing unsaturation (i.e. Ph) lead to improved binding (Me < Et ~ iPr < cPr ~ c Hex << Ph). This can be attributed to the improved lipophilic binding with the WPF shelf on increasing the length of the carbon chain and potential π-stacking with phenylalanine on addition of unsaturation. The greatly improved potency of the Ph over other compounds can again be attributed to π stacking in the WPF shelf.

4.2.1.2 Simple Aryl Sulfonamide Substitution

Results for compound **15** were extremely promising, so it was decided that subsequent modifications would focus on simple substitution of the sulfonamide aryl ring.

The initial SGC SAR for BRPF3 (data unpublished) suggested that electron withdrawing substituents, especially Cl (Hammett coefficients σ_{meta}: 0.37, σ_{para}: 0.23) [68] were preferred in the WPF Shelf. As sequence homology in this area of the protein is very high in the BRPFs, initial substitutions focussed on electron withdrawing groups. [37] Thus compounds **21-23** (Scheme 3) were synthesised where a substitution of the H by a nitrile was investigated around the phenyl ring. A nitrile was chosen as the literature suggested that the Cl to CN (Hammett coefficients σ_{meta}: 0.56, σ_{para}: 0.66) [68] substitution has several beneficial effects:

1. Overall negative potential for the nitrile CN compared with the δ^+ hole for the chloride. This can have a negative impact if the halide is involved in a halogen bond. In addition the nitrile has a significantly larger dipole moment at CN = 4.44 D *vs* Cl = 1.67 D.
2. Nitriles prefer more hydrophilic open binding regions to chlorides. [69]
3. Improved logP (logarithm 1-octanol:water partition coefficient.) PhCN = 1.56, PhCl = 2.84 (ΔLogP = -1.28). Decreasing lipophilicity has beneficial effects on drug like properties such as aqueous solubility, metabolic stability and toxicological outcomes.

   By calculating the LipE (Lipophilic efficiency):

   Lip E = -Log (IC_{50}) – LogP (IC_{50}: concentration required to inhibit 50% of protein)

   Which combines the logP and IC_{50} analysis have shown that nitriles consistently outperform their chloride analogues (Figure 36).

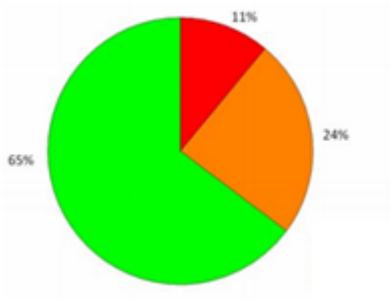
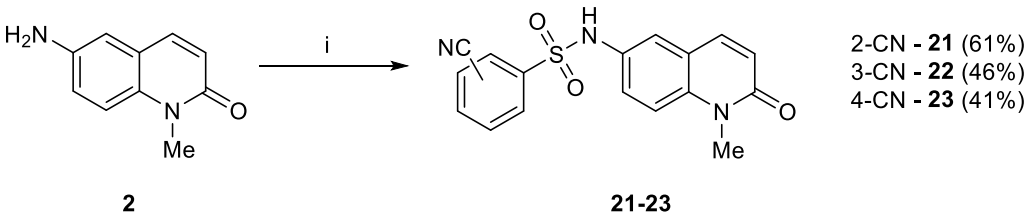


Figure 36. Comparison of LipE for ArCN vs. ArCl in drug candidates from literature and Pfizer internal database. Values are obtained by calculating: ΔLipE = LipE(CN) – LipE(Cl). Green = ArCN is better (ΔLipE > 0.5), Amber = same (0.5 < ΔLipE > -0.5), Red ArCl is better (ΔLipE < -0.5). Reproduced from Jones et al.[69]

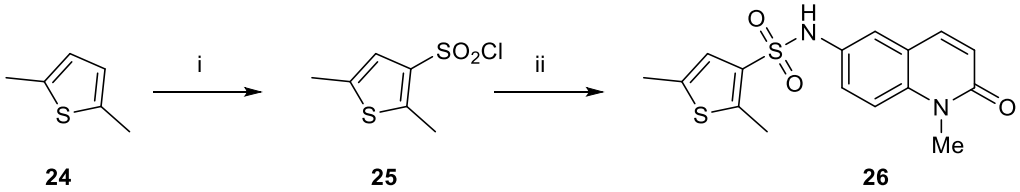
The synthesis of compounds **21-23** initially employed the same conditions as those used for compounds **3, 16-20** (Scheme 3). However, significant difficulties were encountered with purification of the compounds, as on addition of the sulfonyl chloride a dark red solid, containing an unknown contaminant, rapidly precipitated. Attempts at purification were hampered by the exceptionally low solubility of this precipitate (~100 mg not soluble in 10 mL of DMF). Thus, alternative reaction conditions were investigated where precipitation might be avoided to ease purification.



Scheme 3. Reagents and Conditions: i) DMF, RSO_2Cl, Pyridine

Eventually, by changing the solvent to DMF and running the reaction at lower concentration (0.2M) it proved possible to avoid precipitation and concentrate the reaction directly onto celite ® prior to purification by column chromatography.

In addition to the benzonitriles, a 2,5 dimethylthiophene-3-sulfonamide was synthesised as an isostere for the phenyl ring from compound **3**. Addition of sulfuryl chloride to DMF at 0 °C to preform a chloroimidate which was used to introduce a sulfonyl chloride group by electrophilic aromatic substitution on thiophene. Reaction of the crude sulfonyl chloride provided **26** in low yield (Scheme 4).



Scheme 4. Reagents and Conditions: i) DMF, SO_2Cl_2, 0°C *then* **24,** 80 °C, 66%; ii) DMF, **2,** Pyridine, 31%.

Compounds **21-23** and **26** were initially screened against a range of BRDs by Thermal Shift to qualititatively determine compound binding (Table 2).

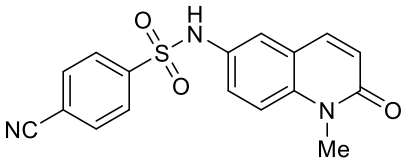
ID	BAZ2A	BRD1	BRD4	BRD9	CECR2	PCAF	BRPF1B	CREBBP	TIF1
21	0.1	0.9	1.3	2.9	0.5	0.2	2.4	0.9	0.9
22	0.2	1.3	1.9	3.5	0.6	-0.1	3.1	0.9	1.1
23	0.3	2.4	1.4	2.3	-0.4	0.1	5.2	-0.1	1.1
26	0.0	1.4	0.9	2.8	0.7	0.2	5.0	0.3	2.1

Table 2. ΔT_m results from sulfonamide investigation (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

With regards to BRPF1 inhibition there is a clear preference, amongst the nitriles, for *para* (**23**) substitution, with the *meta* nitrile (**22**) being slightly more potent than the *ortho* (**21**) compound. The 2,5 dimethylthiophene derivitave (**26**) was of similar potency to both the Ph (**3**) and the *para* nitrile (**23**) compound.

For BRD1 the order of potency is maintained as **23** > **22** > **21**, however there appears to be a much larger selectivity for BRPF1 *vs* BRD9 in compound **23** in comparison to **22.** This is because the ΔT_m for BRD9 for **22** is roughly similar in BRD9 and BRPF1 (Table 2). Thermal shift also suggested that all compounds tested had a similar, but appreciable, level of activity against BRD4(1), but that otherwise binding against non-class IV BRDs was minimal.

In light of this data it was decided to quantitatively quantify potency and selectivity of compound **23** by K_D (Table 3).



MW 339
tPSA 90 Å^2
ClogP 1.96

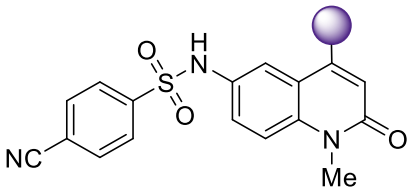
BRD	ΔT_m (°C)[a]	K_D (nM)[b]	Selectivity (× Fold)
BRPF1	**5.2**	**43**	**-**
BRD1	**2.4**	**200**	**4.7**
BRPF3	**n.d**	**1000**	**21**
BRD4 (1)	**1.4**	**2800**	**65**
PCAF	**0.1**	**70,000**	**> 1,000**
CREBBP	**-0.1**	**44,000**	**> 1,000**

Table 3. Biochemical and biophysical evaluation of compound **23**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were

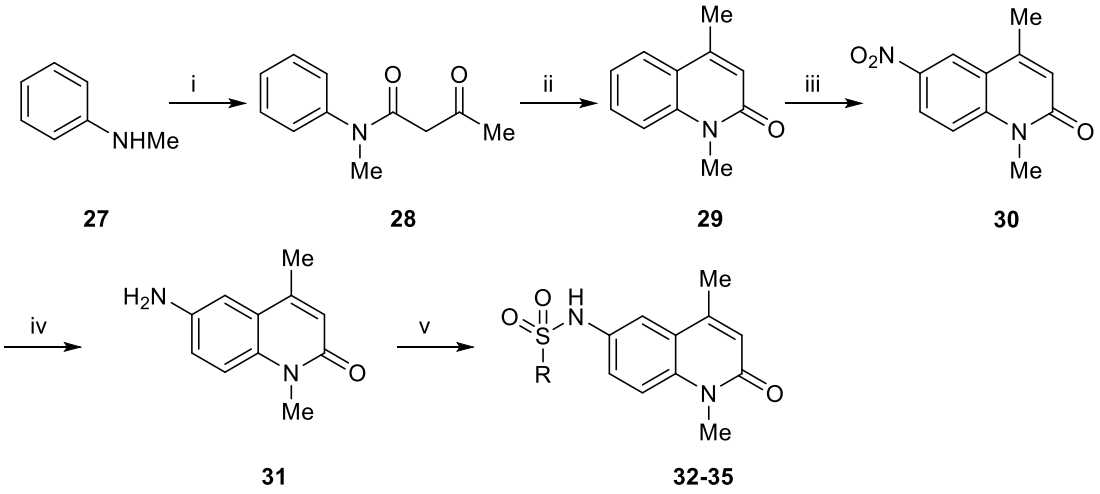
determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates.

The trend in potency suggested by the ΔT_m results was replicated in the K_D's determined. In addition, compound **23** retained the excellent potency against BRPF1 displayed by its *des*-cyano analogue (**3**). Disappointingly, the reasonable activity against BRD4 hinted at in ΔTm corresponds to a 2,800 nM potency and only a 65 fold selectivity window over BRD4. As mentioned previously, this selectivity needed to be greater, as BRD4 is known to have a myriad of biological effects. Activity against other non-class IV BRDs tested was minimal and all activity against PCAF, where the original fragment hit originated from, had disappeared. In addition compound **23** displayed excellent physicochemical, druglike properties, with ClogP of 1.96 limiting lipophilicity induced off-target activity and a TPSA of 90 Å suggesting excellent cell permeability

4.2.2 4-Position Substitution of the Quinolin-2(1*H*)-one A ring



BRD acetyl lysine binding pockets, unlike most protein-protein interaction domains, are deep and hydrophobic. As such, exploiting small lipophilic pockets in this region, that vary from BRD to BRD, can increase both potency and selectivity. To this end, it was decided to synthesise compounds bearing a methyl group at the 4 position, in the hope of serendipitously exploiting such a small lipophilic pocket in BRPF1. The required amine was synthesised according to scheme 5.



Scheme 5. Reagents and Conditions: i) 2,2,6-trimethyl-4H-1,3-dioxin-4-one, xylene, 120 °C, 59%; ii) H_2SO_4, 95 °C, 58%; iii) H_2SO_4, HNO_3, 0–5 °C, 91%; iv) $SnCl_2$, HCl, 78%; v) RSO_2Cl, Pyridine, DMF.

Compound	R	Yield (%)	ClogP
32	Ph(2-CN)	17	2.46
33	Ph(3-CN)	25	2.46
34	Ph(4-CN)	45	2.46
35	2,5-dimethylthiophene	44	3.27

Heating *N*-methylaniline with 2,2,6-Trimethyl-*4H*-1,3-dioxin-4-one in xylene afforded oxobutanamide **28** after attack of the amine and spontaneous loss of acetone. [70] This was then cyclised by heating in concentrated H_2SO_4, followed by precipitation on addition of ice water and filtration. [71] Nitration was carried out regioselectivity as per the literature, [72] and subsequent reduction as for **2** (Scheme 1).

As for amine **2**, **31** was coupled with 2, 3 and 4-cyanobenzenesulfonyl chlorides as well as 2,5-dimethylthiophene-3-sulfonyl chloride using optimised conditions to afford compounds **32-35**.

ID	BAZ2A	BRD1	BRD4	BRD9	CECR2	PCAF	BRPF1B	CREBBP	TIF1
32	-0.3	1.6	0.0	1.5	0.0	-0.4	2.5	0.3	0.6
33	0.2	1.3	1.9	3.5	0.6	-0.1	3.1	0.9	1.1
34	-0.1	1.2	0.6	2.3	-0.3	-0.3	4.3	0.6	0.3
35	-1.4	2.1	0.8	2.7	0.3	-1.0	2.8	0.9	-0.7

Table 4. ΔT_m results from 4-methyl investigation (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

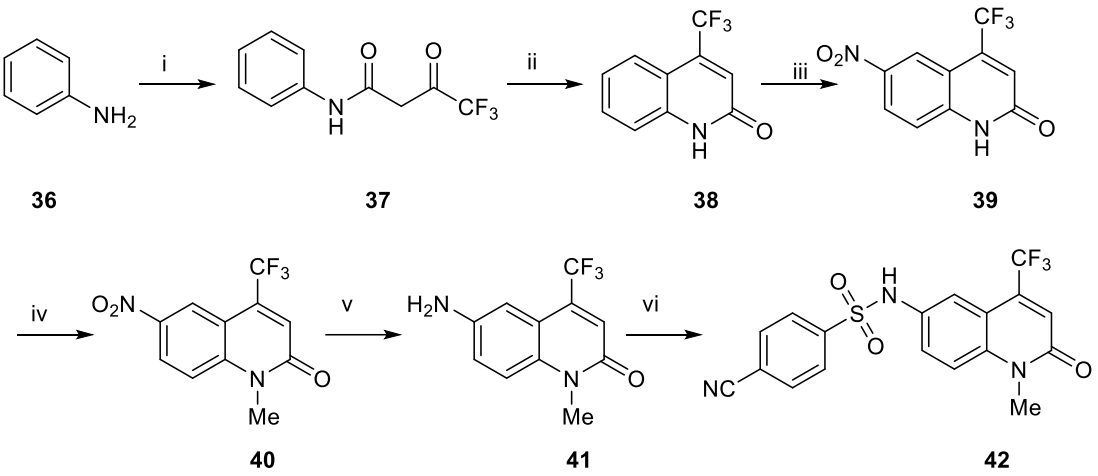
For the analogues of the two most active compounds from the previous series bearing the 4-CN (**34**) and the 2,5-dimethylthiophene-3-sulfonamide (**35**) potency with respect to BRPF1 appeared to drop between 1 and 2 °C on addition of the 4-methyl group. However, activity against the other BRDs remained roughly constant, suggesting lower selectivity for these compounds. For the other analogues (**32:** 2-CN and **33:** 3-CN) the potencies and selectivity profiles appear largely unchanged.

Overall despite the drop for the two most active compounds, the ordering of the compounds in potency against BRPF1 on addition of a 4-methyl group remains unchanged 4-CN>thiophene>3-CN>2CN

In order to verify the apparent drop in potency by thermal shift a K_D was obtained for compound **34** (BRPF1 K_D – 24 nM). This suggested that in contrast to the TS results the introduction of a methyl group could be beneficially to potency (compound **23**, 4 *des*-methyl BRPF1 K_D – 43 nM). On balance, the data is inconclusive as to the effect of adding a methyl group, although it does not appear to be disadvantageous, as initially thought.

4.2.2.1 4-CF_3 Substituted Quinolin-2(1*H*)-one

As the BRPF binding pocket appeared to accommodate the methyl group further SAR was obtained by alteration of the methyl into a trifluoromethyl. This would allow investigation of electron withdrawing groups which are located in proximity to the KAc mimetic.



Scheme 6. Reagents and Conditions: i) Ethyl 4,4,4-trifluoroacetoacetate, 110 °C; ii) H_2SO_4, 95 °C, 38% over 2 steps; iii) H_2SO_4, HNO_3, 0–5 °C, 72%; iv) NaH, MeI, DMF, 94%; iv) $SnCl_2$, HCl, 53%; vi) DMF, Pyridine, RSO_2Cl, 44%.

The required amine was synthesised by first reacting aniline with ethyl-4,4,4-trifluoroacetoacetate at elevated temperatures to form oxobutanamide **37**. [72] Elevated temperatures are required for this step, as reactions at lower temperatures lead to electrophilic attack of the nitrogen at the ketone carbon, which on cyclisation will afford the undesired 1,4 quinolone. Oxobutanamide (**37**) was cyclised in concentrated H_2SO_4, similarly to the cyclisation of compound **28,** to give quinolinone **39**. [72] The nitration followed conditions outlined previously in the literature to give **39** which was methylated, reduced and coupled using standard conditions. [72]

	BRPF1	TIF1	BRD4	BRD9	CECR2	CREBBP
42	3.8	1.4	2.1	3.4	1.0	1.2

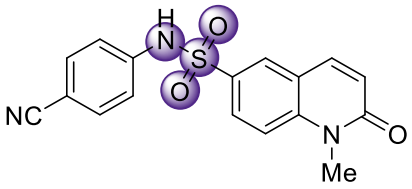
Table 5. ΔT_m results from 4-trifluoromethyl compound **42** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

Substitution of the methyl by trifluoromethyl was not well tolerated by the BRPF binding site, as the BRPF potency dropped ~ 1 °C whilst affinity for other BRDs has increased by TS.

It is possible that the negative results for BRPF1 are due to the very electron withdrawing nature of the CF_3 group (CF_3 Hammett coefficients σ_{meta}: 0.43, σ_{para}: 0.53, CH_3 Hammett coefficients σ_{meta}: -0.07, σ_{para}: -0.17). [68] Located in close proximity to the C2 carbonyl this would withdraw electron density from the carbonyl and have a detrimental effect on compound binding. However, this would not explain why the affinity with respect to BRDs other than BRPF1 was not similarly adversely effected.

It is thus likely that the BRPF binding site was unable to sterically accommodate the increased size of the CF_3 group as well as the other BRDs screened (Taft size parameter E_s CH_3 = -1.24, CF_3 = -2.40). [28]

4.2.3 'Reverse' Sulfonamides



Having confirmed the acitivity of the sulfonamides against BRPF1 we next investigated whether 'reversing' the linker altered binding affinity. It was envisaged that reversing the linker would primarily have two effects on the compounds:

1. Electron density in the quinolinone ring and the KAc mimetic would be reduced. This could reduce compound affinity (Figure 37).

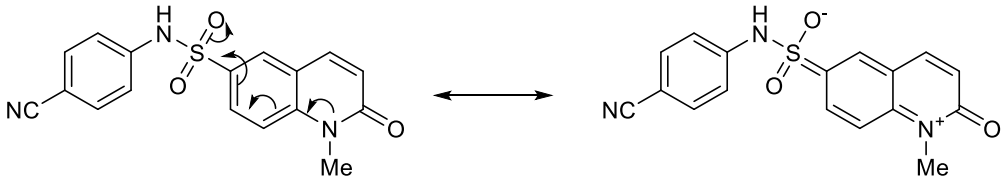
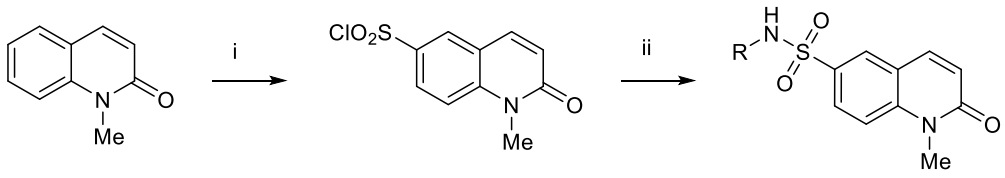


Figure 37. *N* lone pair can resonate into the 6-sulfonamide group reducing electron density in the KAc mimetic.

1. The longer S-C bond (C-N: 147 pm C-S: 182 pm) now linking the quinolinone to the sulfonamide would alter the angle and position at which the sulfonamide aryl ring bends into the WPF shelf. This could be beneficial both in selectivity and potency. [65]

Synthesis of the required sulfonyl chloride was carried out through adaptation of the conditions of Tashima *et al* by heating *N*-methylqinolin-2(1*H*)-one (**1**) in chlorosulfonic acid to afford 6-chlorosulfonyl-*N*-methylquinlin-2(1*H*)-one (**43**) in good yield. [73] Regioselectivity, which could either be at the 6 or the 8 position was confirmed by the presence of a weak *meta*-coupled doublet at 8.28 ppm in the ^{1}H NMR spectrum. The sulfonyl chloride was then coupled in low to good yields with a range of aryl amines (Scheme 7, Table 6).



Scheme 7. Reagents and Conditions: i) HSO_3Cl, 95 °C, 77%; ii) RNH_2, pyridine, DCM.

Compound	R	Yield (%)	CLogP
44	**Ph**	**79**	**2.26**
45	**Ph*N*(Me)**	**71**	**2.11**
46	**Bn**	**86**	**2.36**
47	**PhC_2H_5**	**72**	**2.70**
48	**Ph(4-CN)**	**39**	**1.88**
49	**Ph(3-CN)**	**50**	**1.88**
50	**Ph(2-CN)**	**15**	**1.88**

Table 6 Reverse Sulfonamides synthesised

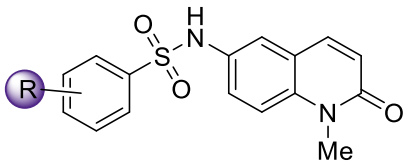
ID	BAZ2A	BRD1	BRD4	BRD9	CECR2	PCAF	BRPF1B	CREBBP	TIF1A
44		1.6					3.5		1.56
46	0.0	1.0	1.7	3.9	0.5	0.2	2.6	0.1	0.7
47	0.1	2.0	0.8	1.2	0.2	-0.1	2.3	0.6	1.1
48	0.5	3.4	1.1	0.9	0.5	0.2	3.0	0.3	1.2
49	0.0	0.7	0.7	2.5	0.7	0.2	1.2	0.4	0.7
50	0	0.1	0.9	1.7	0.6	0.2	0.7	0.2	0.2

Table 7. ΔT_m results for 'Reverse sulfonamides' **46-50** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

As predicted, reversing the sulfonamide linker reduced compound binding to BRPF1, with T_m values decreasing between 1-2 °C. Similarly to the sulfonamide series **21-23** BRPF1 displayed a preference for the *para* substituted compound. K_D determination for compound **48** suggested that the drop in ΔT_m equated to a roughly 50% decrease in potency (**23**: BRPF1 K_D = 43 nM. **48**: BRPF1 K_D = 100 nM).

Despite the drop in potency, it was hoped that the reversal of the linker would increase compound selectivity. This proved unsuccessful, with 4-CN compound (**48**) being equipotent against BRPF1 and BRD1. In addition the Bn analogue (**46**) had a higher affinity for BRD9 than for BRPF1. In general the compounds with the reversed linkers retained potency against BRDs other than BRPF1 whilst losing potency against BRPF1. This equated to a loss in potency and selectivity suggesting that reversing the linker was an unsuccessful avenue of SAR.

4.2.4 Substitution of the sulfonamide ring

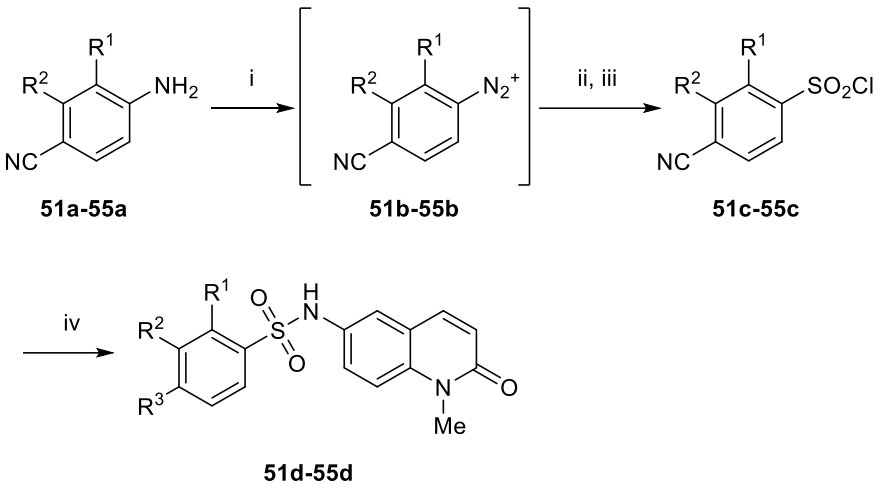


Having determined that the optimal substitution pattern involved a 4-CN substituted sulfonamide, we next decided to investigate sulfonamides with a multiply substituted sulfonamide aryl ring. As the sulfonyl chlorides bearing a nitrile at the 4 position were not commercially available it was first necessary to synthesise the requisite building blocks.

Sulfonyl chlorides can be synthesised by a variety of routes including: oxidation of the corresponding thiol, [74] chlorination of the requisite sulfonic acid, [75] reaction of boronic acids with SO_2Cl equivalents, [76] electrophilic aromatic substitution with HSO_3Cl and the decomposition of diazonium chlorides in SO_2 saturated solutions. [77]

As a consequence of the synthetic tractability and commercial availability of the required amines it was decided to make the sulfonyl chlorides from their respective diazonium chloride salts. Typically this involves saturating AcOH with SO_2, adding catalytic CuCl and then adding the diazonium chloride at low temperature to allow a Sandmeyer reaction to occur.[77] Due to the expense, toxic nature and difficulty involved in handling SO_2 the traditional method was unsuitable for our work. Thus it was decided to adopt the method of Hogan *et al* who had developed a method of reacting diazonium chlorides in aqueous solutions that had been saturated with SO_2 by hydrolysing $SOCl_2$. [78]

The authors reported that this method was well suited to amines bearing mildly electron donating groups, but that addition of strongly electron withdrawing, or electron donating groups would hinder reaction progress. With this in mind a number of optionally substituted 4-CN amines were converted to their sulfonyl chlorides as per Scheme 8.



Scheme 8. Reagents and Conditions: i) Conc HCl 50 °C, 30 mins *then* -5 °C $NaNO_{2(aq)}$ 30 mins; ii) $SOCl_2$, -5 °C *then* ~15 °C 18 h; iii) $SO_{2(aq)}$, CuCl, **51b-55b** , -5 °C 2 h *then* 0 °C - RT 2h; i) DMF, pyridine, **2.**

Compound	R^1	R^2	R^3	Yield SO_2Cl (%)	Yield SO_2NHR (%)	CLogP
51*	F	H	CN	83	79	2.15
52	Br	H	CN	54	71	2.37
53	Me	H	CN	n.d.	12[a]	2.46
54*	H	Cl	CN	69	73	2.59
55	H	Cl	Cl	NA	91	3.48

Table 8. Sulfonyl chlorides and sulfonamides synthesised. *indicates commercially available amine. Other amines were synthesised by adapting literature procedures (see experimental) a. Yield over 2 steps, sulfonyl chloride isolated in a 1:1 ratio with the corresponding chloride and used without further purification

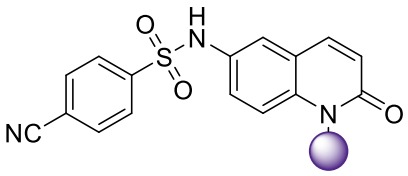
Yields for sulfonyl chloride formation were generally in line with those reported by Hogan *et al*. For the more electron donating 2-Me (Hammett coefficients σ_{meta}: -0.07, σ_{para}: -0.17) the yield was significantly lower, with the sulfonyl chloride being isolated in a 1:1 ratio with the aryl chloride. Routine coupling of the synthesised sulfonyl chlorides provided **51d**-**55d** for biological evaluation against BRPF1.

	BRD1	BRD4	BRD9	BRPF1	TIF
51d	n.d.	n.d.	n.d.	3.6	0.3
52d	3.1	n.d.	0.4	5.2	n.d.
53d	n.d.	n.d.	n.d.	6.5	1.4
54d	-2.1	n.d.	-0.4	4.3	n.d.
55d	2.5	2.8	n.d.	4.9	n.d.

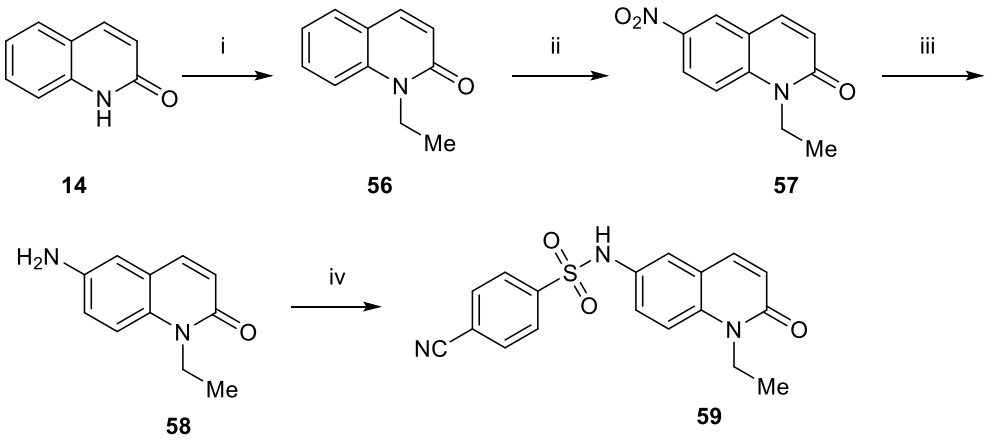
Table 9. ΔT_m results for disubstituted sulfonamides **51d-55d** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

The results indicate that for BRPF potency the most favourable substitution pattern is 2,4 substituted, with more electron donating, lipophlic substituents being preferred at the 2 position (Me>Br>F, in order of ClogP). Interestingly, as measured by TS, the 3Cl-4CN compound (**54d**) is almost equipotent in comparison to the 3,4 Cl (**55d**) compound, but the nitrile appears to impart greatly increased selectivity with decreased lipophilicity. Unfortunately, when assessed by K_D **54d** displayed a potency of 62 nM against BRPF1 and 450 nM against BRD1, suggesting that the nitrile did not impart the selectivity hinted at by TS.

4.2.5 *N* alkyl variation



Typically the BRD binding pocket can accommodate small changes near the warhead binding site, as such it was decided to alter the *N*-alkyl group on the quinolinone. Preliminary investigation focussed on the synthesis of 6-amino-1-ethylquinolin-2(1*H*)-one (**58**) is shown in Scheme 9.



Scheme 9. Reagents and Conditions: i) NaH, EtI, DMF, 66%; ii) H_2SO_4, HNO_3, 0–5 °C; 84%; iii) $SnCl_2$, HCl, quant.; iv) RSO_2Cl, DMF, pyridine, 74%.

Analogously to the synthesis of amine **2**, **14** was first alkylated with NaH in DMF and subsequently nitrated and reduced to give amine **58** in good yield. Niration regioselectivity was confirmed analogously to compound **15.** Subsequent coupling proceeded smoothly to provide sulfonamide **59** for biological evaluation.

Attempts at synthesising an *N*-isopropylated quinolinone were unsuccessful as attempts at alkylation of 6-nitroquinolin-2(1*H*)-one (Synthesised by EB) furnished the *O*-ethylated compound in 82% yield after reaction with 2 iodopropane and crystallisation of the crude reaction material from ethanol.

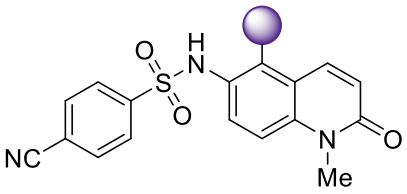
	BRD1	BRD4(1)	BRPF1A
59	1.6	1.8	4.7

Table 10. ΔT_m results for compound **59** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

Substituting the methyl group for an ethyl group appears to have very little effect on compound potency, with K_D's being almost identical between compound **23** (*N*Me) and **59** (*N*Et) (**23**: K_D – 47 nM; **59**; K_D - 43 nM). The K_D values do suggest that ethylating the nitrogen has an advantageous effect in compound selectivity as **59** is 8 fold selective over BRD1 whilst **23** is only 5 fold, however

as these differences are well within the error of the assay there is insufficient data to suggest that this is part of a larger trend. Moreover, the increase in selectivity has come at the expense of a small 0.57 increase in ClogP (ClogP **29** 1.90, ClogP **59**: 2.46).Therefore, altering the methyl group to an ethyl may be beneficial, but would need to be combined with other modifications to be properly investigated.

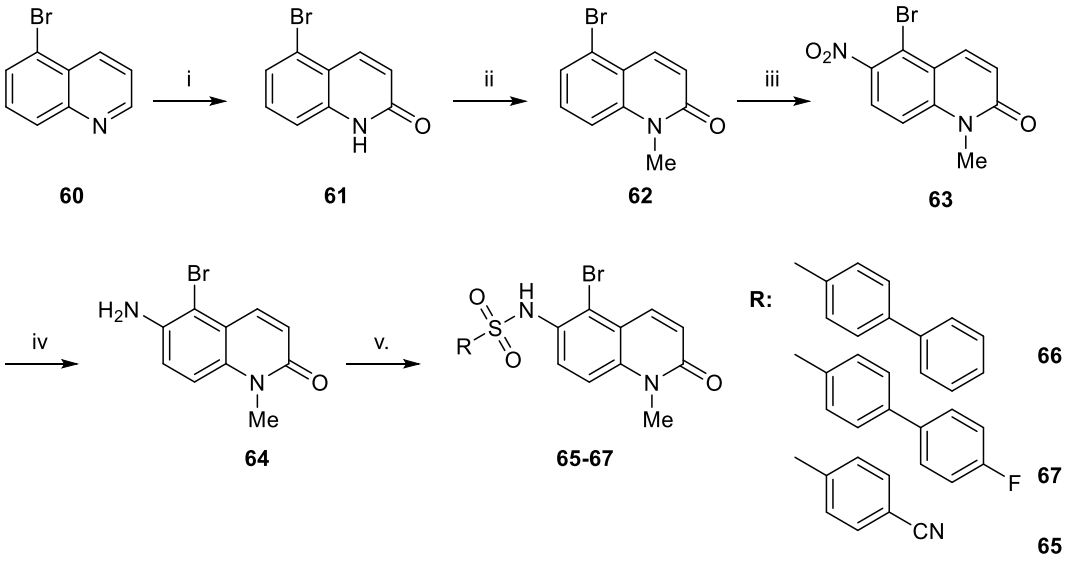
4.2.6 5-Substituted Quinolin-2(1*H*)-ones



Exploration of the quinolinone ring next turned to the B ring by investigating substitution of the 5 position. This position was chosen as a number of appropriately 5-substittued quinolones were readily commercially available, or synthetically tractable.

4.2.6.1 5 Br Substituted Quinolin-2(1*H*)-ones

The first substituent of interest was a bromine, as it was envisaged that the bromine could be used as a synthetic handle to synthesise optionally 5 substuted quinolinones. This could occur *via* either metal medidated coupling, metalation and subsequent reaction, or S_NAr of 6-nitro-5-bromo-quinolinone (**63**).



Scheme 10. Reagents and Conditions: i) *m*CPBA, CH_2Cl_2 *then* NaOH (1.0 M), CH_2Cl_2, 88%; ii) NaH, MeI, DMF, 47%; iii) H_2SO_4, HNO_3, 0–5 °C, 53%; iv) $SnCl_2$, HCl, 69%; v) RSO_2Cl, pyridine, DMF, 28%.

Formation of **61** by was carried out analogously to compound **14** by oxidation and subsequent rearrangement with benzoyl chloride. Nitration, methylation and reduction were also carried out using standard conditions.Regioselectivity of the nitration step was confirmed by comparison of the spectroscopic data for the reduced amine, **64,** with that from the literature. [79] Coupling of the amine provided compound **65** for biological evaluation.

	BAZ2A	BRD1	BRD4	BRD9	CECR2	BRPF1	CREBBP	TIF1A	FALZA
65	-0.2	3.7	1.0	3.0	0.1	6.2	-0.2	0.1	2.5

Table 11. ΔT_m results from compound **65** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

Results appeared very promising, with strong potency against BRPF1 and reasonable selectivity for the BRPFs. The only area of concern was strong activity against FALZ2. As a consequence of the promising results amine **64** was also coupled with 4'fluorobiphenylsulfonyl chlorde and biphenylsulfonyl chloride as *in silico* docking of compound **23** (work carried out Dr Haider, UCL) using published BRPF1 crystal structures suggested that there was more room for optimisation at the 4 position on the sulfonamide ring.

	BRD1	BRD4	BRPF1
65	3.7	1.0	6.2
66	4.1	0.5	5.3
67	4.2	0.4	5.6

Table 12. ΔT_m results from 5- Br sulfonamide investigation (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

Results from addition of the second phenyl ring were unproductive, with compounds being slightly less potent despite the large increases in lipophilicity (ClogPs **65**: 2.31, **66**: 4.28, **67:** 4.44). In order to quantify the potency of **65** of a K_D was determined (K_D BRPF1 – 340 nM).

The value of the K_D was unexpectedly high, therefore we looked to verify the TS data generated. Thus, a new shift was determined at higher concentration (100 μM, TS normally run at 10 μM - Figure 38). Higher concentrations were employed, as any abnormalities in the melting curve are more readily observed. Indeed, inspection of the melting curve for **65** shows a double melting curve, which can be attributed to either compound insolubility, or an abnormal binding mode causing aberrations in the protein unfolding.

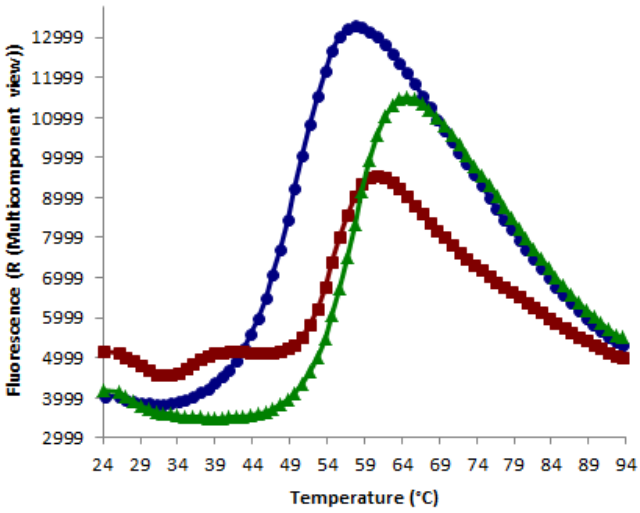


Figure 38. Melting curve for **65** at 100 μM. Blue - control, Red – Compound **65**, Green – Compound **102**

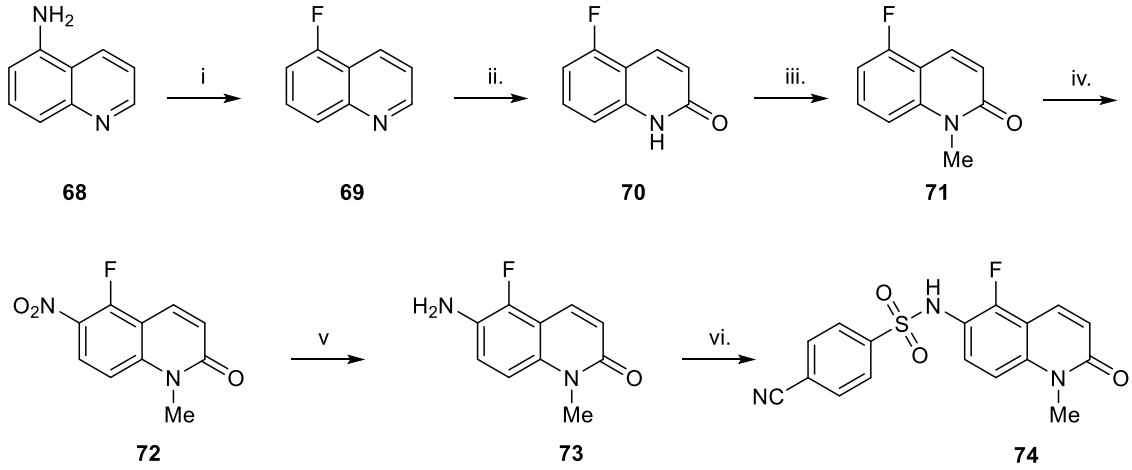
Subsequent to ascertaining that the initial results for **65** were anomalously high, efforts focussed on derivitising the 5 position using the bromine synthetic handle.

Unfortunately, both 5-bromo-1-methyl-6-nitroquinolin-2(1*H*)-one (**62**) and 5-bromo-1-methyl-6-aminoquinolin-2(1*H*)-one (**63**) proved inert to Suzuki coupling conditions ($MeB(OH)_2$, $CsCO_3$ and $Pd(PPh_3)_4$) in attempts to introduce a methyl group. Further attempts at S_NAr reactions of compound **63** were similarly unsuccessful, with compound **63** being inert to both NaOEt and NaOMe at elevated temperatures. Metalation of sulfonamide **65** with BuLi at – 78 °C followed by reaction with iodomethane was also unsuccessful, furnishing a variety of different amines by TLC.

As derivitisation was unsuccessful a selection of 5 substituted compounds were synthesised starting from simple building blocks

4.2.6.2 5-Fluoro Substituted Quinolin-2(1*H*)-ones

As fluorine atoms can be excellent bioisosteric replacements for hydrogen, it was next decided to replace the bromine atom with a fluorine. This should also have the benefit of increasing the solubility of the compound in comparison to bromine, as bromines are notorious for their ability to impart crystallinity.



Scheme 11. Reagents and Conditions: i) HBF_4 *then* Xylene 110 °C, 38%: ii) *m*CPBA, CH_2Cl_2 *then* NaOH (1.0 M), CH_2Cl_2, 82%; iii) NaH, MeI, DMF, 70%; iv) H_2SO_4, HNO_3, 0–5 °C, 43%; v) $SnCl_2$, HCl, 54%. vi) RSO_2Cl, DMF, Pyridine, 80%.

Transformation of the amine into a fluorine by literature Balz-Schiemann reaction afforded 5-fluoroquinoline from 5-aminoquinoline in modest yield. [80] Amine **73** was synthesised *via* the standard reaction sequence depicted in scheme 11. Yields for nitration were slightly lower than expected for the nitration of **71** as multiple regioismers were obtained. Coupling of **73** provided compound **74** for biological evaluation against BRPF1.

	BRD1	BRD4	BRPF1
74	1.4	0.0	2.7

Table 13. ΔT_m results from compound **74** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

Although solubility was noticeably improved, binding affinity had decreased significantly on addition of the electron withdrawing F. This is potentially a consequence of fluorine withdrawing electron density from the quinolinone C2 carbonyl. This would reduce the KAc mimetic's ability to form the key binding interactions and thus reduce affinity.

4.2.6.3 5-OMe Substituted Quinolin-2(1*H*)-ones

As the sulfonamide **74** was hypothesised to be less active as a result of low carbonyl electron density a methoxy group was next introduced. The electron donating ability of the ethereal oxygen would strengthen any interactions that the acetyl-lysine mimetic makes in the protein (Figure 39).

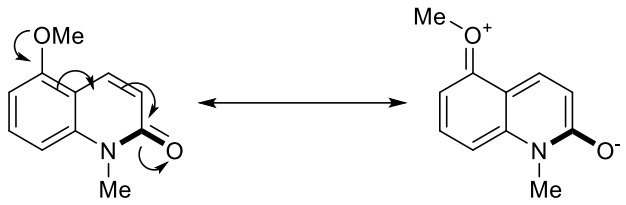
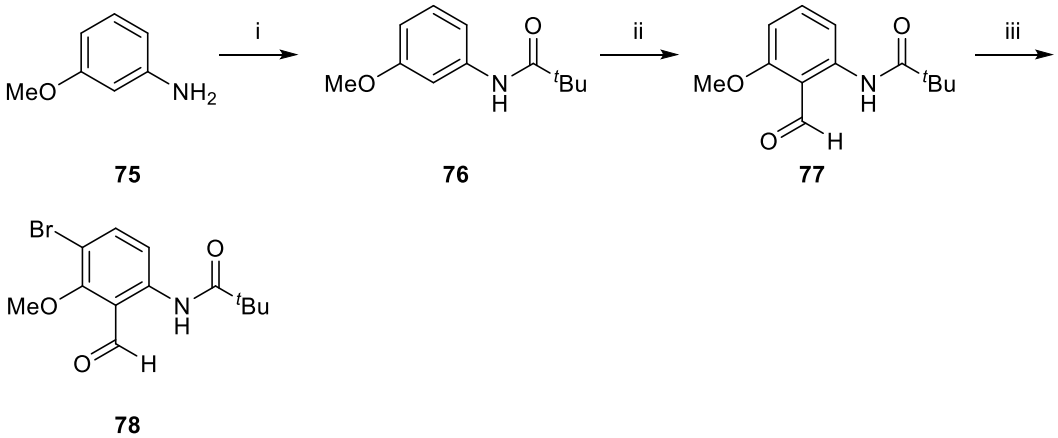


Figure 39. Ethereal oxygen electron density can resonate into the KAc mimetic

As previous routes to 5-methoxyquinolin-2(1*H*)-one (**80**) proved capricious in our hands a synthetic strategy based on Turner *et al*'s synthesis of napthyridinones was employed. [81] *M*-anisidine was first converted into the pivalamide in quantative yield through reaction with the acid chloride in pyridine and DCM. [82] With both the methoxy and pivalamide groups acting as *ortho* directing metalation groups for the 2 position, metalation with n-BuLi and reaction with the aldehyde equivalent DMF provided *N*-(2-formyl-3-methoxyphenyl)pivalamide (**77**) in good yield. [83] Initially it was decided to introduce a bromine as a synthetic handle at the 4 position at this point in the synthesis as the large pivalamide group would sterically hinder electrophilic substitution *ortho* to the amide.



75 **76** **77**

78

Scheme 12. Reagents and Conditions: i) Pivaloyl chloride, pyridine, CH_2Cl_2, quant.; ii) *n*-BuLi, THF, 0 °C *then* DMF, 73%; iii) NBS, DCM, 0 °C, 22%.

Reaction of **77** with NBS in DCM provided the desired regioisomer **78**, as confirmed by a NOESY interaction between the *tert*-butyl group and the hydrogen at the 6 position (Figure 40). However, as the yield for the reaction was very low it was decided to construct the quinolinone A ring before electrophilic substitution was carried out.

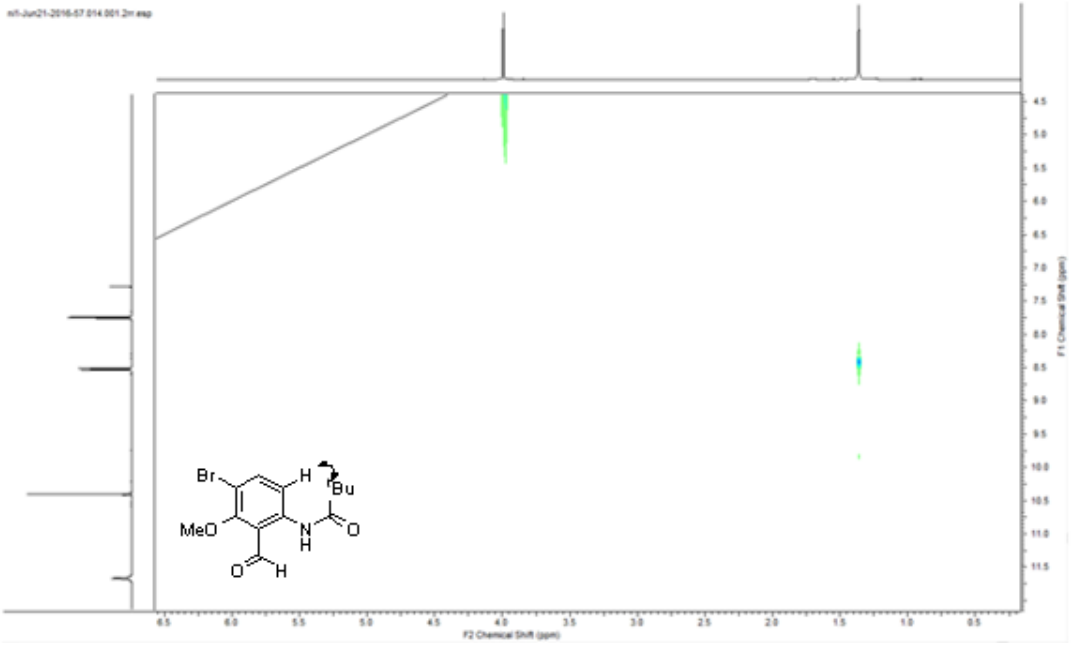
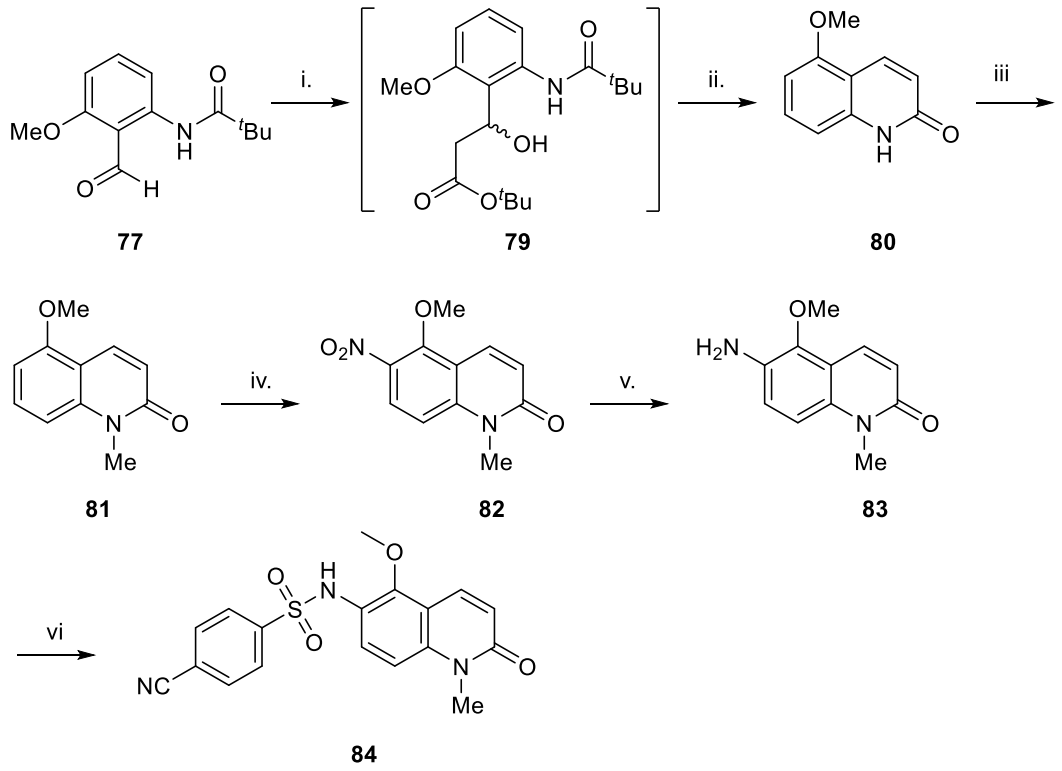


Figure 40. NOESY interaction between the ^{t}Bu group at 1.36 ppm and C$_6$*H* at 8.52 ppm

Thus, compound **77** was treated with the pregenerated enolate of *tert*-butylacetate to form the ***β***-hydroxyketone (**79**). **79** was then deprotected, cyclised and dehydrated by heating in a 1:1 mixture of HCl (3 M) and 1,4 dioxane to give 5-methoxyquinolin-2(1*H*)-one (**80**) in excellent yield.



Scheme 13. Reagents and Conditions: i) *t*-BuAc, LDA, -78 °C, ii) HCl (3 M) : 1,4 dioxane (1:1), reflux, 90% over 2 steps; iii) NaH, MeI, DMF, 64%; iv) H_2SO_4, KNO_3, 0–5 °C, 14%; v) $SnCl_2$, HCl; vi) DMF, Pyridine RSO_2Cl, 22% over 2 steps.

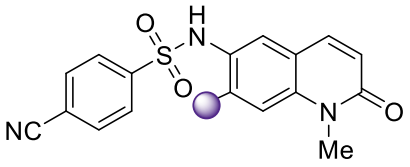
Disappointingly, the yield for nitration of **81** using stoichiometric KNO_3 to control mono *vs* di-substitution was poor, with the desired regioisomer being isolated in 12%. As for previous nitration steps electrophilic substitution could either happen at the 6 or 8 positions of the quinolinone ring. Nitration regioselectivity was confirmed by NOESY interaction of the amino group with the methoxy methyl group following reduction. Despite the poor yield for nitration, reduction and subsequent coupling by standard conditions afforded the desired sulfonamide (**84**) for biological evaluation against BRPF1.

	BRD1	BRD4	BRPF1
84	1.5	0.2	3.0

Table 14. ΔT_m results from compound **84** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

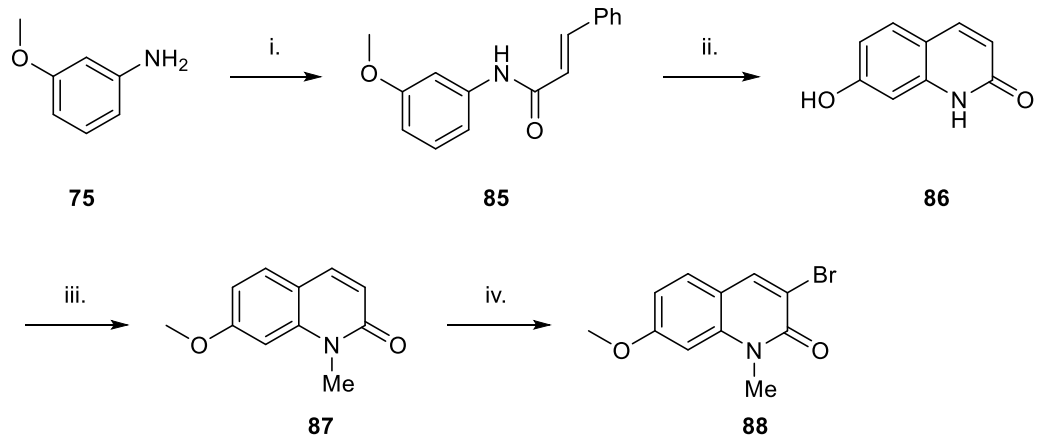
Despite the increased electron density of the quinolinone ring, binding affinity was still poor (BRPF1 T_m – 2.95 °C). As results for modification of the 5-position were not tolerated, leading to lower binding affinity, further optimization in this area was abandoned.

4.2.7 7-Substituted Quinolin-2(1*H*)-ones



4.2.7.1 7-OR Substituted Quinolin-2(1*H*)-ones

Efforts at compound optimisation were next directed towards the 7 position. *In silico* screening by our collaborators at the SGC suggested that introduction of a methyl ether at the 7 position could improve compound potency. The initial synthetic strategy towards the synthesis of amine **93** is outlined in scheme 14.



Scheme 14. Reagents and Conditions: i) Cinnamoyl chloride, K_2CO_3, Acetone, quant; ii) $AlCl_3$, chlorobenzene, 58%; iii) NaH, MeI, DMF, 54%; iv) NBS, DMF, 92%.

Biphasic amidation conditions gave compound **85** in quantitative yield which was cyclised *via* an unusual intramolecular Friedel Crafts reaction which, according to the literature, involves the loss of a phenyl anion in order to aromatise the quinolin-2(1*H*)-one A ring (Figure 41). [97] The concurrent methyl ether deprotection carried out in the reaction gave quinolinone **86** in modest yields, which contained small amounts of the 5-hydroxyquinolin-2(1*H*)-one (7%) regioisomer after crystallization from ethanol.

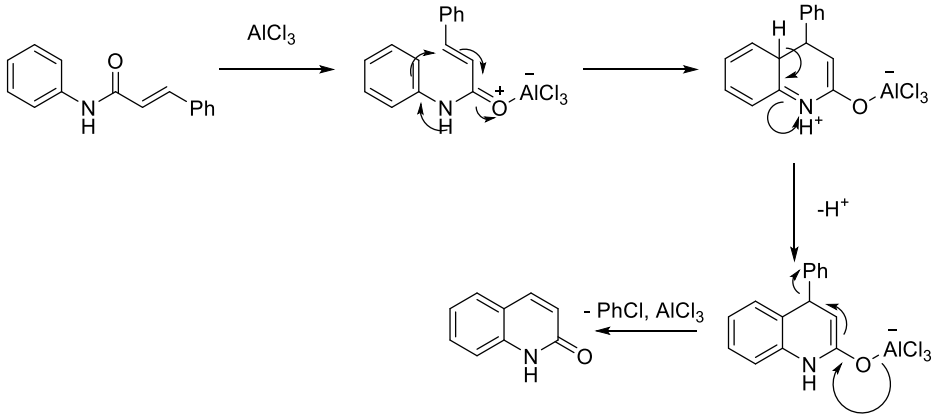


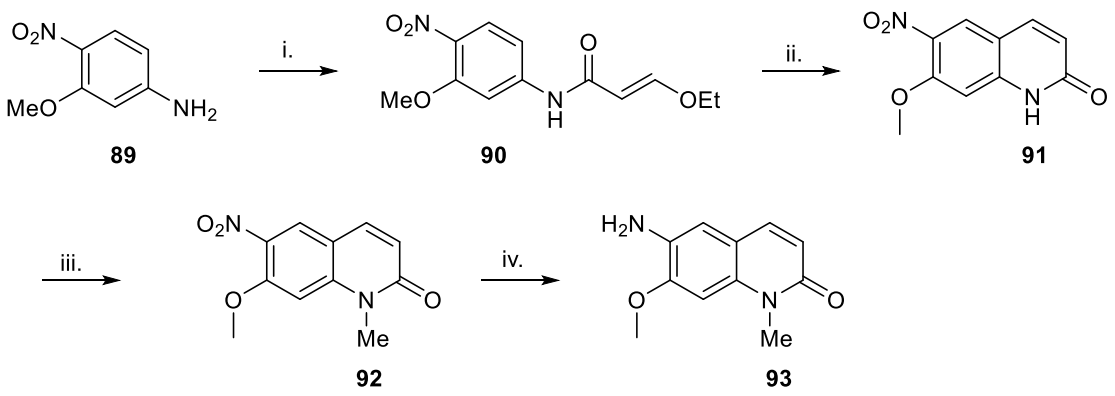
Figure 41. Proposed mechanism for the cyclisation of *N*-(Phenyl)cinnamides [97]

Double methylation using NaH and iodomethane yielded compound **87** in moderate yield. However, attempts at nitration were unsuccessful as it proved very difficult to control mono *vs* di substitution

even using nitronium tetrafluoroborate, a mild source of NO_2^+. In addition, as the products were exceptionally insoluble, column chromatography to separate product regioisomers proved unsuccessful.

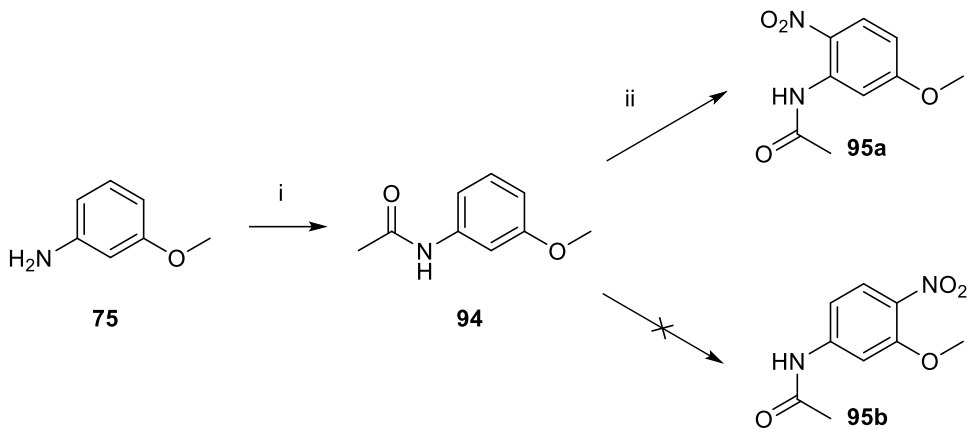
An alternative approach was thus adopted which would involve the bromination and either subsequent amination and coupling, or direct metal mediated cross coupling with the primary sulfonamide to provide the desired compound. Unfortunately bromination with NBS in DMF proceeded to give the undesired 3-Br isomer (**88**) in excellent yield. Regioselectivity was confirmed by HSQC/HMBC assignment and the lack of a characteristic C$_3$*H* signal at ~ 6.4 ppm with a strong *ortho* coupling. Instead the signal at 6.79 ppm has a weak *meta* coupling of 2.3 Hz. Investigation of DCM and acetic acid as alternative solvents had little effect on regioselectivity.

Searching of the literature suggested that it would be possible to synthesise **93** by the sequence illustrated in scheme 15. [84]



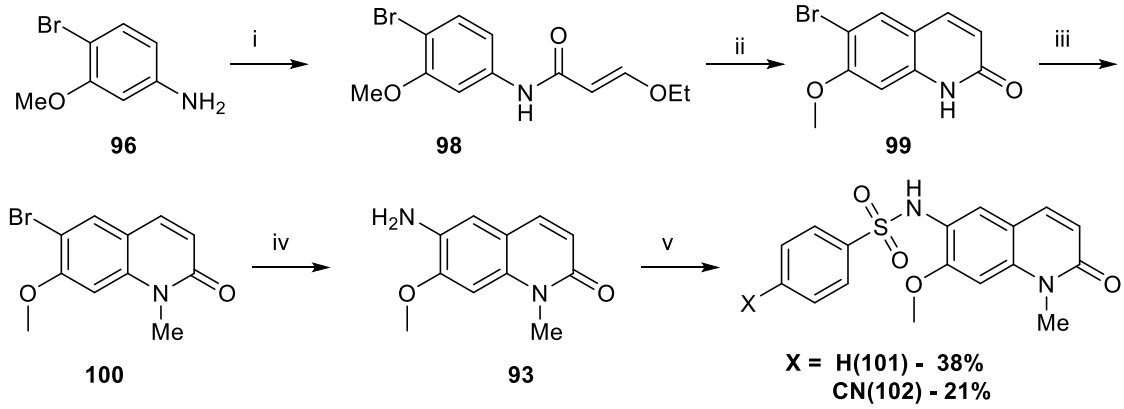
Scheme 15. Reagents and Conditions: i) Ethoxyacraloyl chloride, pyridine, CH_2Cl_2; ii) H_2SO_4; iii) K_2CO_3, MeI, DMF; iv) Pd/C, H_2

Efforts were thus made to synthesise amine **89,** which according to literature could be synthesised, all be it in 20% yield, from *m*-anisidine using conditions outlined in scheme 16. [85] Acetylation with acetic anhydride in acetic acid gave **45** in quantitative yield. [86] However, reproducing literature nitration to give the desired regioisomer proved unsuccessful in our hands, instead exclusively isolating a 41% yield of **95a**. [85]



Scheme 16. Reagents and Conditions: i) AcOH, Ac_2O, quant,; ii) HNO_3, AcOH, Ac_2O 0 °C

Since amine **96** was commercially available the synthesis was adapted to give Scheme 17.



Scheme 17. Reagents and Conditions: i) Ethoxyacraloyl chloride (**97**), pyridine, CH_2Cl_2, 59%; ii) H_2SO_4, quant; iii) NaH, MeI, DMF, 56%; iv) cat. Cu_2O, NH_4OH (28–30% NH_3), NMP μw, 110 °C; v) RSO_2Cl, pyridine, DMF.

2-ethoxyacraloyl chloride (**97**) was synthesised following the literature procedure in 2 steps from ethyl vinyl ether and oxalyl chloride followed by decarbonylation on heating. [87] Reaction of the crude acid chloride with amine **96** gave amide **98** in moderate yields. Cyclization in concentrated H_2SO_4 yielded **99** in quantitave yield which was methylated under standard conditions to give 1-methyl-6-brome-7-methoxy-quinolin-2(1*H*)-one (**100**) in modest yields.

Initial attempts at amination by adapting the procedure of Xu *et al* proved unsuccessful as bromide **100** proved inert to these conditions. [88] In order to inhibit starting material precipitation on addition of NH_4OH the ratio of NMP was increased to aid solubilisation. Disappointingly even after heating at 110 °C overnight bromide **100** still failed to react.

Xu *et al* had also investigated the use of microwave irradiation for the amination of substitutionally inert halides with some success. Gratifyingly, on application of microwave conditions the reaction proceeded smoothly to the desired amine (**93**). Unfortunately even after column chromatography and prolonged drying under high vacuum it proved impossible to remove NMP from amine **93**.

Thus, the crude amine was coupled in low yields with the 2 most promising sulfonyl chlorides using standard conditions to give the sulfonamides **101** and **102** for biological evaluation.

ID	BAZ2A	BRD1	BRD4	BRD9	CECR2	BRPF1B	CREBBP
101	n.d	1.9	n.d	3.5	n.d	5.3	n.d
102	-0.1	0.80	-0.3	0.9	0.0	5.6	-0.2

Table 15. ΔT_m results from compounds **101** and **102** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1 Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

Whilst both compounds (**101** and **102**) showed similar levels of potency against BRPF1 compared to one another and their *des*-methoxy analogues (**2** and **23**) the selectivity of **102** against the other class IV BRD family members and other BRDs was greatly improved. This is potentially a function of the sulfonamide N*H* forming an intramolecular hydrogen with the ether oxygen and orientating the sulfonamide in a position favourable for BRPF binding (Figure 42).

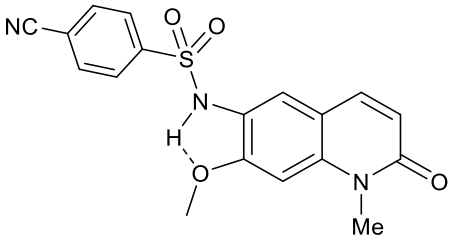
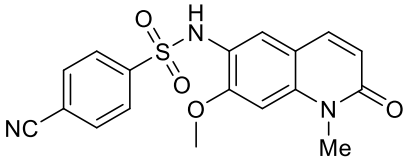


Figure 42. Compound **102** showing the internal hydrogen bond between the ethereal oxygen and sulfonamide nitrogen.

With these promising TS results quantitative K_D's were obtained (Table 16).



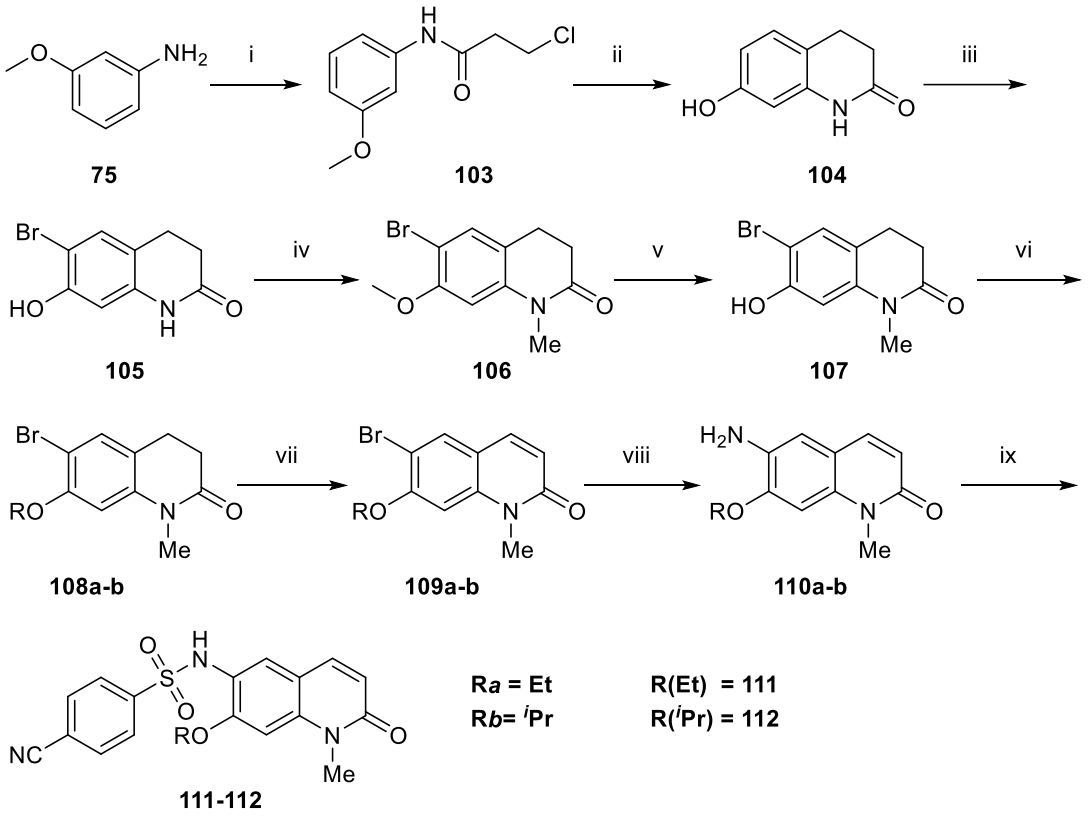
MW 369
tPSA 100 Å^2
ClogP 1.96

BRD	ΔT_m (°C)[a]	K_D (nM)[b]	Selectivity (× Fold)
BRPF1	**5.6**	**53**	**-**
BRD1	**0.8**	**520**	**9.8**
BRPF3	**n.d.**	**2200**	**42**
BRD9	**0.9**	**2700**	**51**
BRD4(1)	**-0.3**	**> 10,000**	**> 200**
CREBBP	**-0.2**	**> 10,000**	**> 200**

Table 16. Biochemical and biophysical evaluation of compound **102**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

102 possessed an excellent probe profile in terms of selectivity and potency. In addition, the ClogP of 1.90 combined with PTSA 100 Å^2 should impart favourable off-target selectivity and cell permeability. However, lack of compound solubility would hinder widespread biological application. Compound **102**'s insolubility was ascribed to its planarity and the intramolecular hydrogen bond between the sulfonamide N*H* and the ethereal oxygen (Figure 42).

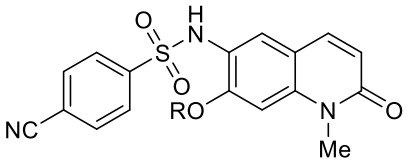
It was hoped that extending the alkyl ether at the 7 position would detrimentally impact crystal packing, thus improving compound solubility whilst retaining the intramolecular hydrogen bond for selectivity. The required amines for the 7 alkyl ether analogues were synthesised according to scheme 18.



Scheme 18. Reagents and Conditions: i) 3-chloropropionyl chloride, K_2CO_3, Acetone 0 °C, 79%; ii) $AlCl_3$, DMA, 150 °C, 54%; iii) NBS, DMF iv) NaH, MeI, DMF, 73%; v) BBr_3 (1.0 M in heptane), THF, -78 °C to RT, 61%; vi) NaH, RI, DMF, a: quant, b: 63%; vii) DDQ, DCE, reflux, a: quant, b: quant; viii) cat. Cu_2O, NH_4OH (28–30% NH_3), NMP μw, 110 °C ix) RSO_2Cl, pyridine, DMF, a: 17% over 2 steps, b: 24% over 2 steps

Amidation was carried out analogously to the formation of compound **85** in high yield. Cyclisation was achieved by heating the amide in DMA with $AlCl_3$ under argon at 150 °C to give the desired 7-hydroxy-3,4-tetrahydroquinolinone (**104**) after crystallisation from ethanol. [89] Subsequent

literature bromination, employing NBS in DMF afforded the desired 6-bromo isomer, as evidenced by the 2 singlets in the ^{1}H NMR spectrum. [90] Subsequent double methylation was carried out as per compound **85**. Reaction of methyl ether **106** with BBr_3 at -78 °C furnishing phenol **107** in reasonable yield. Deprotonation of the phenol with NaH and reaction with the requisite alkyl iodide furnished the appropriate 7-alkoxy tetrahydroquinolinone (THQ) in good to excellent yield. The THQ was oxidised to the quinolinone by heating a mixture of the THQ with freshly crystallized DDQ in DCE at reflux. Whilst purified DDQ was not critical to reaction success it was observed that the yield was slightly higher when the purified reagent was employed. With the 6-bromo-7-alkoxy-1-methylquinolinones in hand amination and subsequent coupling were carried out as per compound **100** to afford the sulfonamides (**111**-**112**) in low yield.



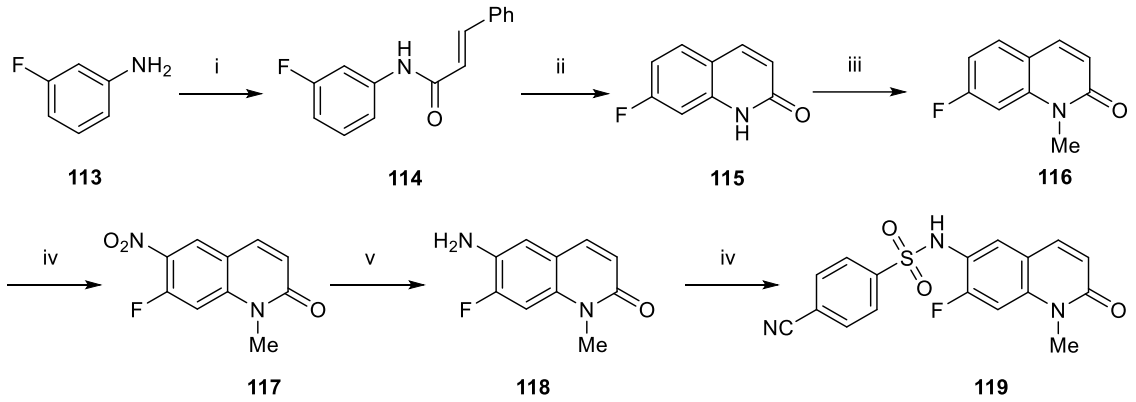
R	BRPF1 ΔT_m (°C)[a]	BRPF1 K_D (nM)[b]	CLogP
Me (102)	8.7*	53	1.98
Et (111)	0.4	55	2.50
^{i}Pr (112)	1.1	36	2.80

Table 17. Biochemical and biophysical evaluation of compounds **103, 111** and **112**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates* Compound was assayed again on the same plate to provide more meaningful comparison, value is ~2 °C higher than previously recorded.

After the negative results from the TS assay it was hypothesised that the ether at the 7 position was exploiting a small lipophilic pocket that was unable to accommodate a larger alkyl group. Given the low T_m values it was thus unusual that the K_D's determined were similar to that determined for **103**. Although there may not always be a direct correlation between the TS and K_D data the magnitude of the difference suggests that one of the assays was erroneous.

4.2.7.2 7-Fluoro Substituted Quinolin-2(1*H*)-ones

Replacement of various substituents with a fluorine atom had previously proved unsuccessful in terms of BRPF1 potency. However, noticible improvements in solubility had been observed. Thus, in an effort to improve the undesirably low solubility of **103** , the ether was replaced with a fluorine.



Scheme 19. Reagents and Conditions: i) Cinnamoyl chloride, K_2CO_3, Acetone, 93%; ii) $AlCl_3$, chlorobenzene, 61%; iii) NaH, MeI, DMF, 87%; iv) KNO_3, H_2SO_4 0 °C, 34%; v) $SnCl_2$•$2H_2O$, HCl, 39%; vi) RSO_2Cl, Pyridine, DMF, 83%.

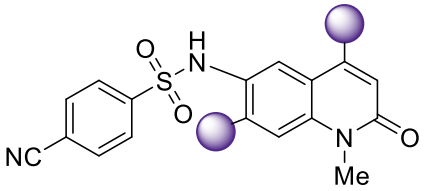
In a similar synthetic sequence to scheme 14, 3-fluoroaniline (**113**) was coupled to form cinnamide **114** which was subjected to intramolecular cyclisation. **115** was methylated, nitrated to give the desired 6-nitro isomer **117**. This was confirmed by ^{1}H NMR, with a pair of doublets for C_3H and C_4H as well as doublets for C_5H and C_8H. The alternative 8-nitro isomer would have displayed a doublet of doublets for the C_6H proton. Reduction and coupling under standard conditions afforded sulfonamide **119** for testing against BRPF1.

ID	BRD1A	BRD4A	BRPF1B
119	1.8	0.7	3.8

Table 18. ΔT_m results from compounds **119** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

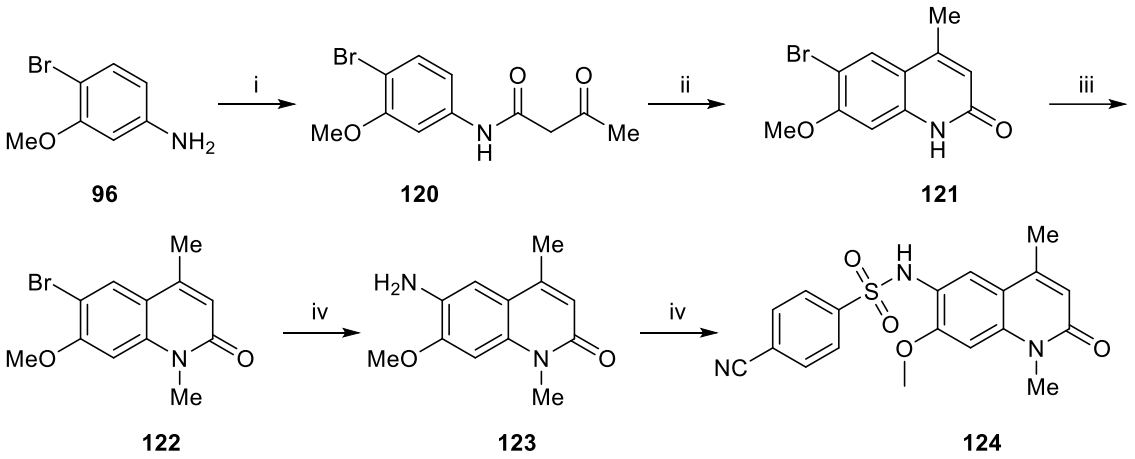
Substitution of the 7 ether did prove efficacious in terms of solubility, with **119** being soluble at 100 mM in DMSO which **103** was not. Despite this, the substitution pattern was not investigated further, as the compound displayed lower potency and off-target selectivity. Similarly to compound **74** this could be a function of lower KAc mimetic electron density, or the lack of intramolecular hydrogen bond to preorientate the compound in a mode favourable for BRPF1 binding.

4.2.8 4,7 Substituted Quinolin-2(1*H*)-ones



As it was thought that the ether variation at the 7 position was unproductive, it was decided to reintroduce the methyl group at the 4 position. It was hoped that this would retain compound selectivity, whilst addressing the solubility issues associated with **103**. A merged synthetic strategy

from schemes 5 and 17 was employed for the synthesis of 7-methoxy-6-amino-1-methylquinolin 2(1*H*)-one (**124**) which is depicted in scheme 20.



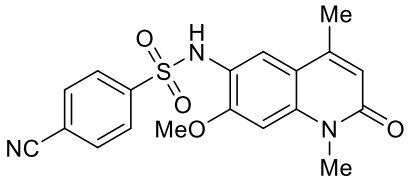
Scheme 20. Reagents and Conditions: i) 2,2,6-trimethyl-4H-1,3-dioxin-4-one, xylene, 120 °C, 66%; ii) Polyphosphoric acid, 100 °C, 96%; iii) NaH, MeI, DM, 52%; iv) cat. Cu_2O, NH_4OH (28–30% NH_3), NMP, μw, 110 °C; iv) RSO_2Cl, DMF, Pyridine, 20% over 2 steps.

Formation of the oxobutanamide was consistant with previous reactions, although cyclization conditions with sulfuric acid were unreproducible, with none of the desired quinolinone being isolated. In contrast, by changing the acid source to polyphosphoric acid (PPA) a quantitative yield of **121**, with none of the undesired 6-bromo-5-methoxy-4-methylquinolin-2(1*H*)-one was obtained, as evidenced by the two singlets for protons C_5H and C_8H. PPA was chosen as it has a long history in the literature of being an excellent condensation, dehydration and cyclisation reagent. [91] Ensuing methylation and amination were carried out as previously described to yield the amine which was coupled under standard conditions to provide compound **124**.

ID	BRD4A	BRD9A	CECR2A	CREBBPA	BRPF1	TIF1A
124	0.4	1.1	0.3	0.2	7.1	2.2

Table 19. ΔT_m results from compound **124** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.Heat map is a scale from Green = low ΔT_m - inactive to Red = high ΔT_m – active.

Although it was not possible to collect TS data on BRD1 and BRPF3, the general selectivity profile of **103** was maintained on addition of the methyl group at the 4 position, whilst maintaining the potency against BRPF1.



MW 383
tPSA 100 Å^2
ClogP 2.47

BRD	ΔT_m against BRPF1 (°C)[a]	K_D against BRPF1 (nM)[b]	Selectivity (124) against BRPF1	Selectivity (102) against BRPF1
BRPF1	7.1	57	-	-
BRD1	n.d.	1800	32	9.8
BRPF3	n.d.	9300	163	42
BRD9	1.1	390	15	51

Table 20. Biochemical and biophysical evaluation of compound **124**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

Determination of the K_D's indicated that **124** was more selective than **102** with respect to BRD1 and BRPF3, but less selective for BRD9. The promising K_D's merited further investigation, thus an ITC K_D (experiment carried out by Cynthia Tallant at the SGC) was determined. Disappointingly this gave a value of 423 nM against BRPF1, suggesting that the competition assay K_D's determined by DiscoverX were overestimating binding by approximately 10 fold.

Despite the disappointing affinity, it was observed that **124** possessed marginally better solubility than **102**. **102** required sonication to dissolve and then was observed to precipitate from DMSO after 7 days, which was not the case for **124** (~ 100 mM). The added soluibility allowed for the determination of a cocrystal structure of **124** with BRPF1.

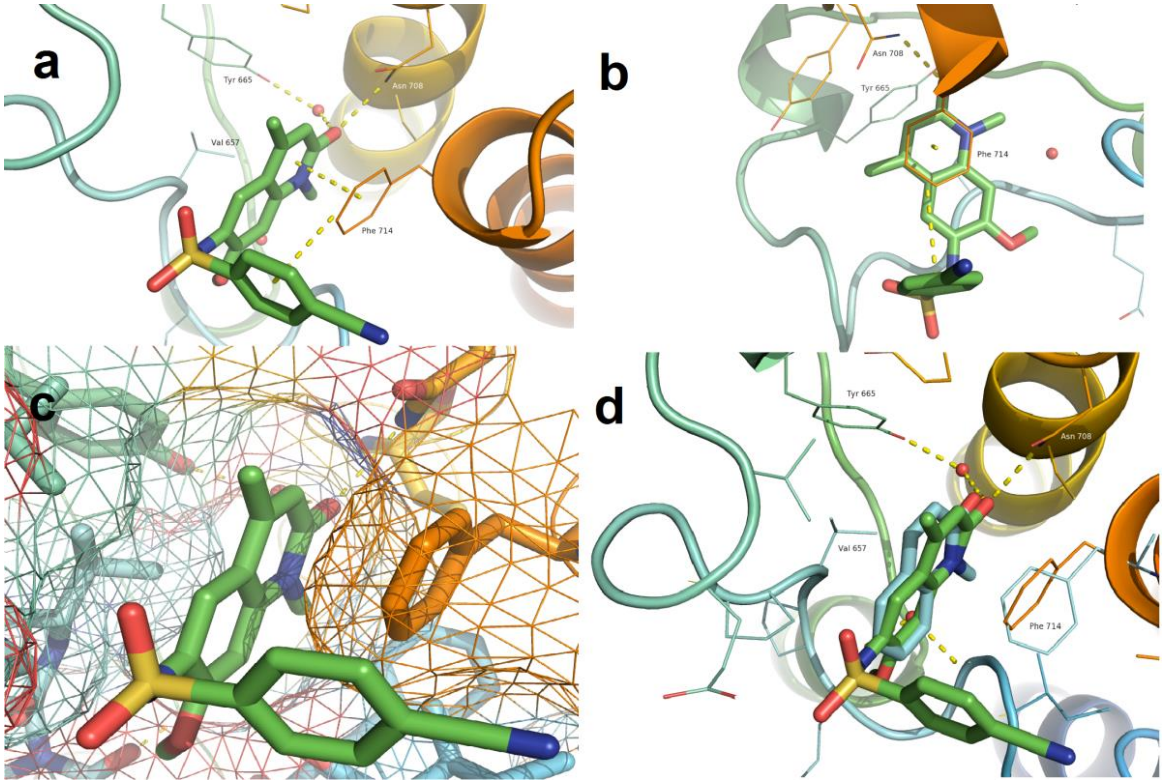


Figure 43. Cocrystal structure of compound **124** with BRPF1 (1.65 Å) . Data obtained by Cynthia Tallant at the SGC. Diagram created in PyMOL. a. **124** in BRPF1 highlighting the binding interactions between the quinolinone A ring and F714 and the KAc mimetic with N708 and W665 b. View of **124** in BRPF1 looking down F714, highlighting the π-stacking between F714 and the quinolinone A ring. c. Surface of BRPF1 showing the pocket available at the 3-position. d. Superimposed crystal structures of **124** and 1-methylquinolin-2(1*H*)-one (**14**- light blue) showing same binding mode, but 'tilting' of F714 in **14** structure (light blue)

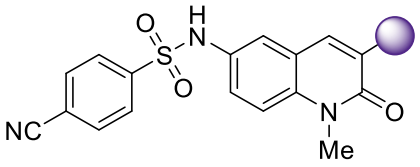
The crystal structure highlights a number of key interactions:

1. As expected, the quinolinone C2-carbonyl acts as an acetyl lysine mimetic. The amide forms a hydrogen bond (2.8 Å) with Asn 708. This is the only direct polar interaction of the molecule (Figure 43a).
2. The C2-carbonyl interacts with the conserved Tyr 665 *via* a water mediated H-bonding network (Figure 43a).
3. Quinolinone A-ring forms parallel, face-centered π-π stack with Phe 714 'gatekeeper' residue (3.6 Å) (Figure 43b).
4. Quinolinone A-ring sandwiched between Phe 714 and Val 657 (Figure 43a).
5. Benzonitrile forms perpendicular, T-shaped C-H-π interaction with Phe 714 (5.0 Å) (Figure 43a).
6. Overlaying a cocrystal structure of the fragment bound to BRPF1 (obtained later in the project) illustrated that elaboration into the sulfonamide caused Phe 714 to tilt such that it can now π-stack with the quinolinone A ring (Figure 43d).

By obtaining a cocrystal structure of compound **124** with BRPF1 it was possible to shift from systematic to a structure guided SAR approach. Through analysis of the key inhibitor interactions a number of further areas of optimisation were prioritised.

1. The quinolinone was orientated such that the 4-methyl group was pointing into open space, not picking up any interactions. Further investigation of this group was unlikely to provide the extra potency required.
2. Analysis of the polar surface of the binding site suggested C3 position modification might be able to exploit a small lipophilic pocket (Figure 43c).
3. Aromaticity of the quinolinone A-ring and the sulfonamide ring was important to maintain the π stacking with phenylalanine.
4. Increasing the electron density of the benzonitrile aromatic ring might strengthen the T-shaped π bond.

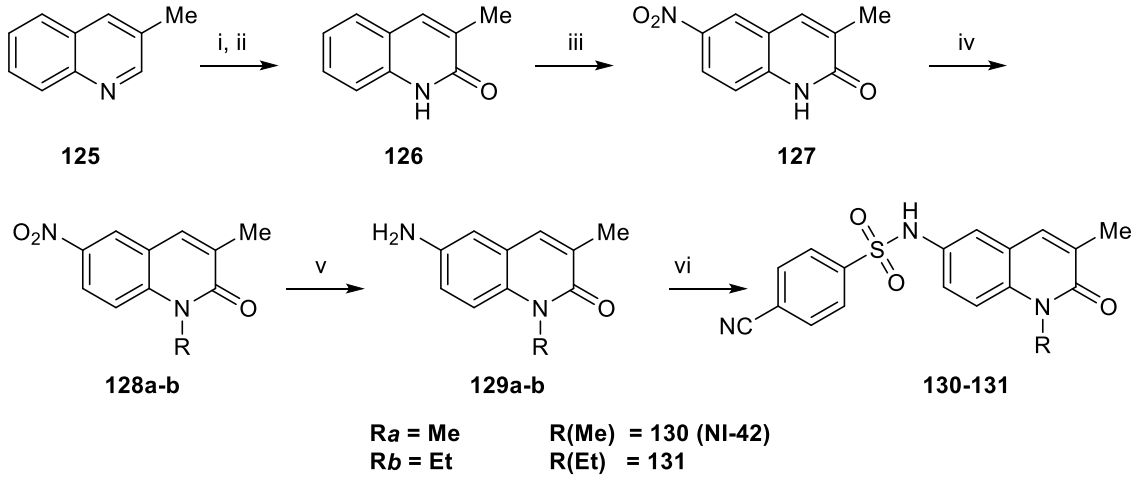
4.2.9 3-Substituted Quinolin-2(1*H*)-ones



Further attempts at investigating the sterically demanding area of the binding pocket next focussed on altering the 3 position, as the cocrystal structure of **124** and BRPF1 showed that there was a small lipophilic pocket in this area.

4.2.9.1 3-Me Substituted Quinolin-2(1*H*)-ones

As the lipoplic pocket available appeared to be quite small the first subsituent investigated was a methyl group. The required quinolin-2(1*H*)-one was synthesised according to Scheme 21.



Scheme 21. Reagents and Conditions: i) *m*CPBA, CH_2Cl_2 ii. Benzoyl chloride, NaOH (1.0 M), CH_2Cl_2, 68% over 2 steps; v) H_2SO_4, HNO_3, 0–5 °C; 77% iii) NaH, RI, DMF, a: 55%, b: 83%; v) Fe, THF:H_2O:EtOH, 80 °C, a: 74%, b: 62%; vi) RSO_2Cl, Pyridine, DMF a: 37%, b: 48%.

The required 3-methylquinolin-2(1*H*)-one (**125**) was synthesised as per the synthesis of scheme 1, by oxidation of the 3-methylquinoline with *m*CPBA and subsequent rearrangement of the *N*-oxide with benzoyl chloride. In contrast to scheme 1, the quinolinone was first nitrated to afford **127**, [92] as this would facilitate corroboration of the data generated from the investigation of the *N*-alkyl group. The undesired 8-nitro isomer was not observed in the ^{1}H NMR as there was no doublet of doublets for the C_6 proton. Alkylation under standard conditions yielded **128a-b** in reasonable to good yield, however issues were encountered with the reduction step. Application of the previously employed $SnCl_2$ lead to a complex mixture of products, so the conditions of Fish *et al* were instead adopted to yield the amine (**129a-b**) in moderate yield. [92] Sulfonamidation afforded compounds **130** (henceforth referred to as **NI-42**) and **131** for biological evaluation.

Initial evaluation of **NI-42** by TS highlighted excellent potency against, and selectivity for, the class IV BRDs (Figure 44). Whilst **NI-42** did not meet the selectivity criteria for a BRPF probe, it was

found to be almost equipotent against all class IV BRDs, further profiling was carried out as it was envisaged that it could be employed as a pan class IV BRD probe.

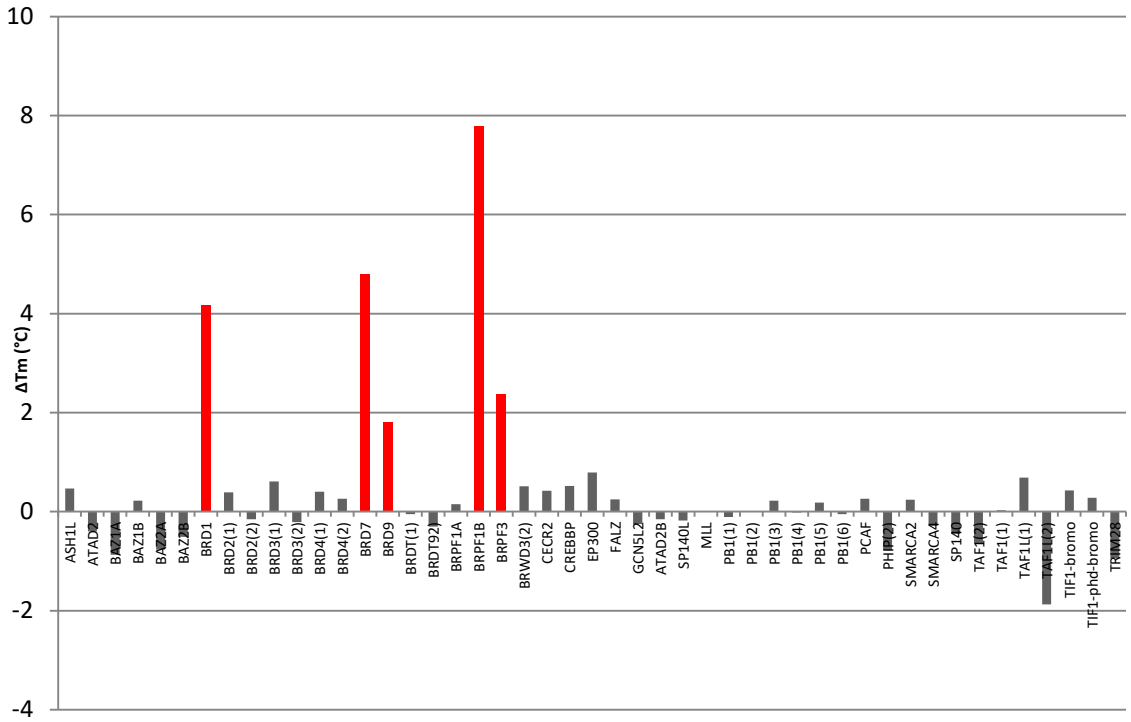
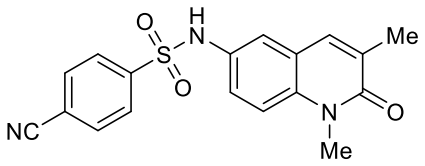


Figure 44. Graph showing bromonome screening of **NI-42** by TS. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 3

The first thing of note, is the lower values of TS in comparison with other compounds screened. This is a result of differences in assay execution when profiling one compound against the entire bromonome. When many compounds are screened the individual compounds are added to the wells containing the BRD of interest, whereas when profiling a single compound the BRD is instead added to the well containing the compound. The net effect of this reversal of addition is that the results obtained from the BRD wide screen tend to be lower, and more accurate as a result of a more consistent compound concentration.

NI-42 was extremely selective for the class IV BRDs, with the highest ΔT_m outside the class IV family being 0.69 °C recorded for TAF1L, with previous TS data from the SGC indicating that these values corresponded to K_D values greater than 1 μM. With this data in hand K_D's were determined to further confirm compound selectivity.



MW 383
tPSA 90 Å^2
ClogP 2.46

BRD	ΔT_m (°C)[a]	Dx K_D(nM)[b]	Dx Selectivity (× Fold)	ITC K_D(nM)[c]	ITC Selectivity (× Fold)
BRPF1	7.8	7.9	-	40	-
BRD1	4.2	48	6.0	210	5.25
BRPF3	2.4	260	33	943	24
BRD9	1.8	310	39		
BRD7	4.8	82	10		
ATAD2A	-0.4	> 10,000	> 200		
ATAD2B	n.d.	> 10,000	> 200		
BRD4(1)	0.4	4,000	> 200		

Table 21. Biochemical and biophysical evaluation of **NI-42**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates. c. ITC K_d's determined by Cynthia Tallant at the SGC, n = 1.

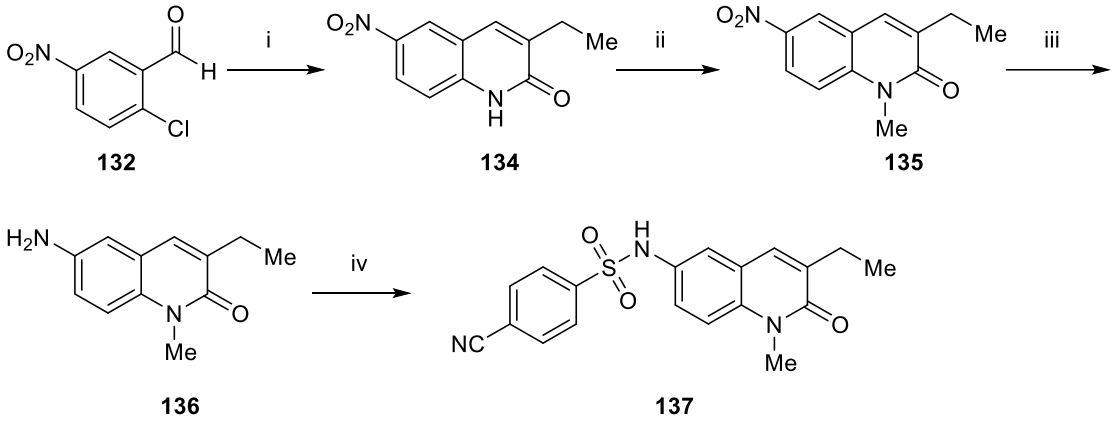
The data from the TS assay was corroborated by the both DiscoverX and ITC K_D's , with **NI-42** being potent against the Class IV's, but inactive against BRDs outside this family. There was a surprisingly high K_D for BRD7, for which TS data can be misleading due to this BRDs very low melting temperature (melts partially at RT). Also of note is that the drop in potency from the Dx platform to the ITC was far lower (~5 fold) than that for compound **124**.

For the *N*-ethylated compound (**131**) the BRPF1 ΔT_m of 5.5 suggests that, in combination with the favourable 3-methyl group, an ethyl group is not well tolerated by the BRPF binding site. However, K_D determination (BRPF1 K_D: 15 nM) suggested that the drop in potency was not as large as the TS value suggests, corroborating the data from compound **59**.

As envisaged, the introduction of a methyl group at the 3 position of the A ring was exploiting a small hydrophobic pocket near the KAc binding site. As a consequence of this, the binding the potency of **NI-42** is improved approximately 5-fold in comparison to its *des*-methyl compound **23** (BRPF1 K_D **23**: 43 nM **NI-42**: 7.9 nM).

4.2.9.2 3-Et Substituted Quinolin-2(1*H*)-ones

We next investigate the size of the hydrophobic pocket at the 3 position by synthesising the 3-Ethyl substituted quinolinone (Scheme 22).



Scheme 22. Reagents and Conditions: i) $NaHSO_4$, 4,4-dimethyl-2-propyl-4,5-dihydrooxazole (**133**), Xylene, NMP RT - 110 °C *then* 110 - 210 °C, 89%; ii) NaH, MeI, DMF, 79%; iii) Fe, THF:H_2O:EtOH, 80 °C; iv) RSO_2Cl, Pyridine, DMF, 17% over 2 steps.

For the synthesis of the required nitroquinolinone (**134**) an alternative route, based on that of Wu *et al* was employed. [93] This involved the reaction of 2-chloro-4-nitrobenzaldehyde (**132**) with 4,4-dimethyl-2-propyl-4,5-dihydrooxazole (**133**). The reaction proceded by formation of the enamine from the oxazole, followed by nucleophilic attack on the aldehyde carbon. Dehydration is followed by the oxazole carrying out S_NAr at the carbon bearing the chlorine substituent, followed by ring opening. Loss of a proton followed by ether hydrolysis furnishes the desired 3 alkyl quinolinone (Figure 45).

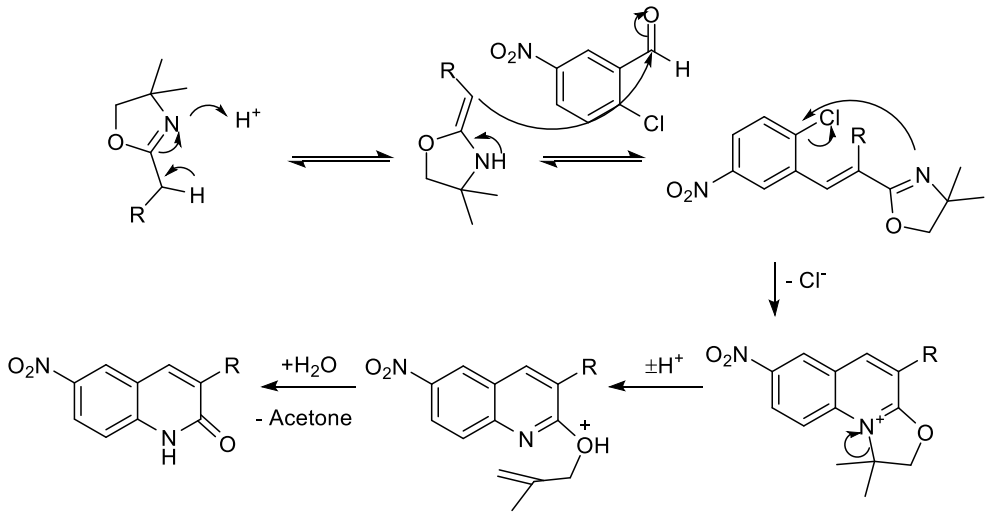


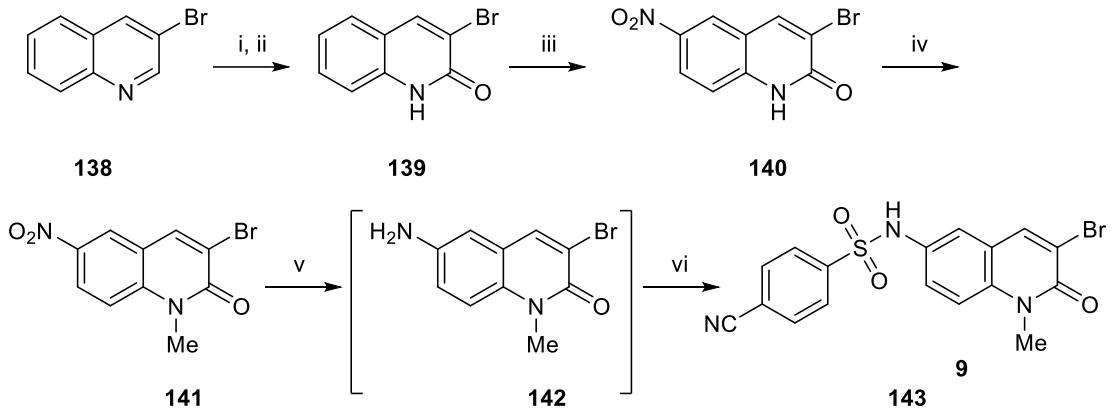
Figure 45. Proposed mechanism for quinolinone formation from 4,4-dimethyl-2-alklyl-4,5-dihydrooxazoles and 2-halo-4-nitrobenzaldehydes

Methylation, reduction and coupling were carried *via* the standard procedures to provide compound **137** for biological evaluation. **137** recorded a ΔT_m of 4.7 °C and a K_D of 180 nM against

BRPF1 suggesting that the hydrophobic pocket at the 3 position was unable to accommodate larger alkyl groups.

4.2.9.3 3-Br Substituted Quinolin-2(1*H*)-ones

Increasing the 3 position substituent size from a methyl to an ethyl group resulted in a drop in compound potency, suggesting that the binding pocket was unable to accommodate the larger ethyl group. A compound with a 3-Br was next synthesised as a marginally smaller (Br Taft size parameter E_S: -1.16, Me E_S: -1.23) [65], more lipophilic replacement for the methyl. Synthesis of the desired sulfonamide was carried out as per scheme 23.

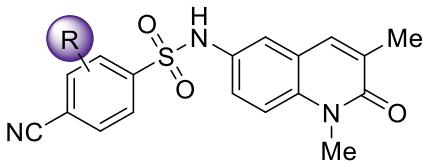


Scheme 23. Reagents and Conditions: i) *m*CPBA, CH_2Cl_2 ii) Benzoyl chloride, NaOH (1.0 M), CH_2Cl_2, 79%; iii) H_2SO_4, HNO_3 0-5 °C, 51%; iv) NaH, MeI, DMF, 33%; v) $SnCl_2$, HCl; vi) RSO_2Cl, Pyridine, DMF, 48% over 2 steps.

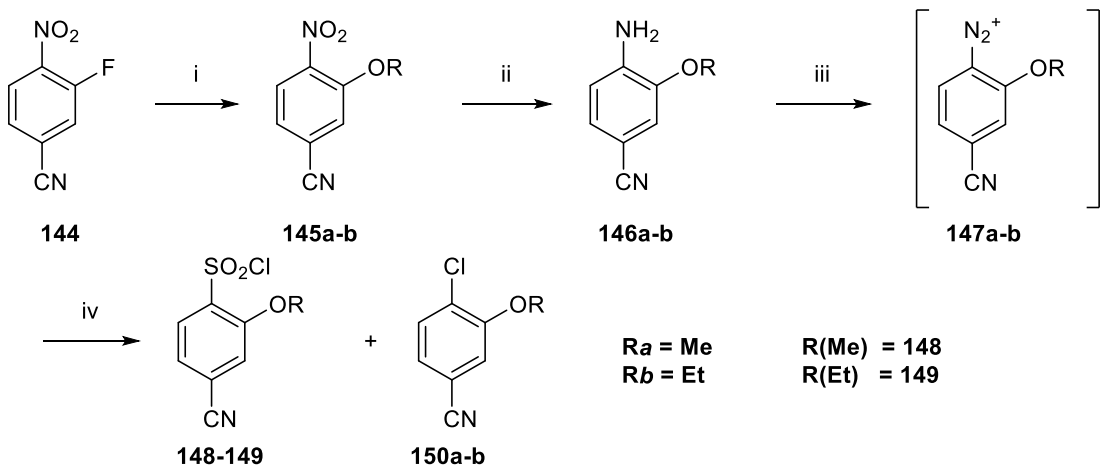
Oxidation and rearrangement employing standard conditions provided 3-bromoquinolin-2(1*H*)-one, which was nitrated to afford **141** using HNO_3 in H_2SO_4. The 6-nitro isomer regioselectivity was confirmed analogously to **127**. Methylation, reduction and coupling of the crude amine afforded sulfonamide **143** for biological evaluation against BRPF1.

Sulfonamide **143** recorded a K_D of 7.9 nM against BRPF1, equipotent to the 3-Me analogue (**NI-42**). However, the complete profile of **NI-42** was superior to the 3-Br sulfonamide (**143**) as it displayed lower lipophilicity (3-Br ClogP – 2.88, 3-Me ClogP – 2.46) and hence improved lipophilic efficiency.

4.2.10 Substitution of the Sulfonamide ring II



Having determined the ideal substitution of the quinolinone ring to be 1,3-dimethyl-6-aminoquinolinone, further sulfonamide substitution was now investigated utilising this core. The most promising substitution pattern from investigation with the 6-amino-1-methylquinolin-2(1*H*)-one (**2**) core involved the electron donating methyl group at the 2 position. As the co-crystal structure of **124** corroborated this SAR, highlighting a T-shaped π stacking interaction between the sulfonamide aryl ring and Phe 714, it was decided to prioritise the synthesis of 2-methoxy-4-cyanobenzenesulfonyl chloride (**148**). This would retain the 4-cyano group for selectivity, whilst introducing a 2-methoxy group for its electron donating abilities.



Scheme 24. Reagents and Conditions: i) NaOMe, THF, 0 °C, a: quant, b: 87%; ii)$SnCl_2$ MeOH, reflux, a: 74%, b: 43%; iii) Conc HCl 50 °C, 30 mins *then* -5 °C $NaNO_{2(aq)}$ 30 mins; iv) $SOCl_2$, -5 °C *then* ~15 °C 18h *then* CuCl, X, -5 °C 2h *then* -5 °C - RT 2h.

Substitution of the fluorine by S_NAr with NaOR provided **145a-b** in excellent yields which were reduced in modest yield in alkyl alcohol at reflux with $SnCl_2$ to furnish the required amines (**146a-b**). Unfortunately, application of the previously employed conditions for conversion of the amine to the sulfonyl chloride were unsuccessful for compound **146a**, failing to yield any of the desired compound. This was in agreement with the literature observations of poor conversion for electron donating substituents, [78] thus optimisation of the various reaction parameters was required (Table 22).

Conc. $SOCl_2$ (mL/ mmol of NH_2)	Reaction concentration (mL of H_2O/ mmol NH_2)	CuCl (mol%)	Temp (°C)	Yield (%)[a]
0.4	2	0.1	-5	-
0.6	2	0.1	-5	12 (1:1)[a]
0.6	1	0.1	-5	-
0.6	2	0.1[b]	-5	17 (1:1)[a]
0.6	2	0.1[b]	-15	0 - 33[c] (2:1)[a]
0.6	2	0.1[b]	-15	27 (4:2:1)[a,d]
0.6	2	0.1[b]	-15	0[e]

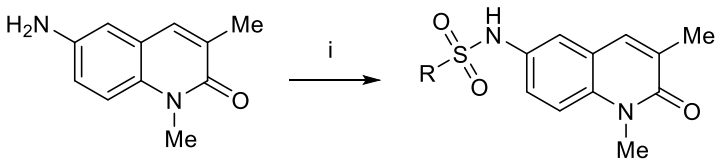
Table 22. Optimization of the Sandmeyer transformation of amine **146a** into 2-methoxy-4-cyanobenzenesulfonyl chloride (**148**). a. ratio of sulfonyl chloride:aryl chloride b. indicates use of freshly purified CuCl c. reaction run under these conditions 4 times. Yields = 0%, 0%, 10% and 33%. All other reactions n=1 . d. optimised conditions used for 2-ethoxy-4-cyanoaniline (**146b**). e. optimised conditions used for 2-methoxy-4-chloroaniline (**151)**, n = 2.

Efforts initially focussed on encouraging a faster reaction, as in previous successful reactions on addition of the diazonium salt the reaction was visibly rapid (the reaction would darken and then would return to pale green in ~15 minutes, followed shortly thereafter by precipitation of the product). Increasing the amount of $SOCl_2$, in the hopes of increasing the SO_2 concentration, proved partially successful isolating the sulfonyl chloride in low yield. Doubling the reaction concentration proved unsuccessful, whilst using freshly purified CuCl appeared to have a small benefit. The most successful modification involved decreasing the temperature to -15 °C during diazonium addition, followed by allowing the reaction to stir at -5 °C and then RT. This suggests that the limiting factor may be instability of the corresponding diazonium salt. Disappointingly even using optimised conditions the reaction proved capricious, failing to yield any sulfonyl chloride in half of the attempts.

Attempts to synthesise 2-ethoxy-4-cyanobenzenesulfonyl chloride (**149**) using the optimised conditions were successful, however the yield was low and an unidentified third product was formed during the reaction. Despite this sufficient quantities of the sulfonyl chloride were synthesised for coupling.

Further application of the conditions to 2-methoxy-4-chloroaniline (**151**) failed to yield any of the desired sulfonyl chloride in two attempts.

With the required sulfonyl chlorides in hand a selection of sulfonamides were synthesised in order to investigate the effect of T-shaped π stacking on potency in the 3-methyl-6-sulfonamide series.



129a **152-154**

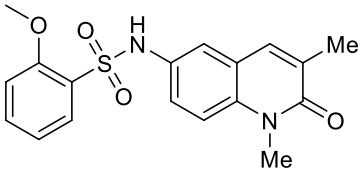
Scheme 25. Reagents and Conditions: i) RSO_2Cl, DMF, Pyridine

Compound	R Group	Yield (%)	ΔT_m against BRPF1 (°C)[a]	K_D against BRPF1 (nM)[b]	CLogP
152	CyHex	79	8.6	31	2.89
NI-42	Ph(4-CN)	37	9.7	7.9	2.46
153	Ph(2-OMe)	65	11.5	9.0	2.18
154 (NI-57)	Ph(2-OMe 4-CN)	24	12.3	4.0	2.08

Table 23. Biochemical and biophysical evaluation of compounds **152-154**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates.

Overall the K_D and TS data indicate a general trend that increasing the electron density of the sulfonamide ring increases compound affinity. However, the differences between compounds **152, NI-42** and **153** are all within the error of the assay, thus absolute ranking of the compounds is not possible. Despite this, addition of the nitrile from compound **153** to **154** (henceforth referred to as **NI-57**) did appear to be beneficial in both assay formats

As Fish *et al* had synthesised **153** as an inhibitor of BRD4 compound selectivity for BRPF1 was next assessed.[92]



MW 358
tPSA 75 Å^2
ClogP 2.18

BRD	ΔT_m (°C)[a]	K_D (nM)[b]	Selectivity by K_D (× Fold)
BRPF1	7.8	9	-
BRD1	n.d.	130	14
BRD4(1)	n.d.	600	66

Table 24. Biochemical and biophysical evaluation of compound **153**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates.

In terms of potency against BRPF1 and selectivity versus BRD1 **NI-42** and **153** had almost identical profiles. However, **153** was also a moderately potent inhibitor of BRD4 (BRD4(1) K_D: 600 nM), providing only a 66 fold selectivity window, much lower than the > 200 fold selectivity of **NI-42**. Given the importance of BRD4 selectivity this compound was not profiled further.

4.2.10.1 NI-57

Since introduction of the nitrile had previously been successful in imparting selectivity for BRPF1 (cf **101** *vs* **102**), following on from the promising TS and K_D data, **NI-57** was profiled by TS against the available bromonome (Figure 46).

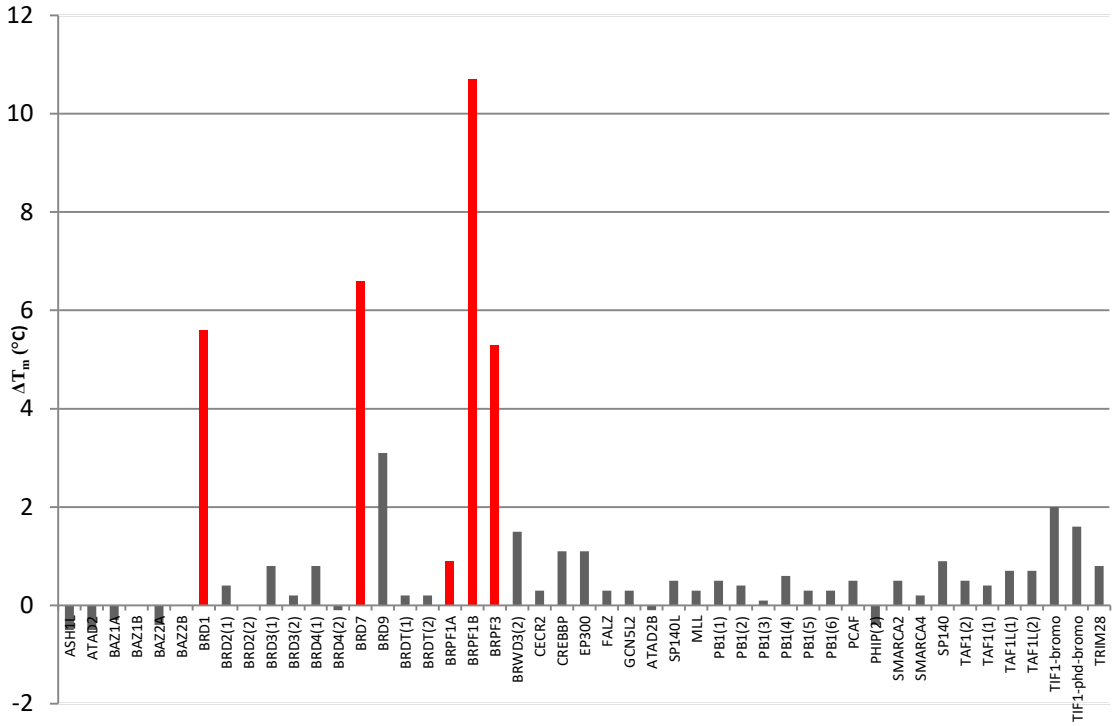


Figure 46. Graph showing bromonome screening of **NI-57** by TS. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 3. Red = Class IV BRD

As for **NI-42**, **NI-57** proved very selective for the class IV BRDs by thermal shift, although unlike **NI-42** there were indications that the **NI-57** may be sufficiently selective for the BRPFs to be considered a BRPF probe. (ΔT_m BRPF1-BRD9 **NI-57**: 7.6 °C, **NI-42**: 5.98 °C, ΔT_m BRPF1-BRD7 **NI-57**: 4.1 °C, **NI-42**: 3.0 °C). Therefore, **NI-57** was further profiled by DiscoverX K_D against the entire BRD class (Table 25).

Determination of the selectivity by DiscoverX K_D's indicated that **NI-57** met the SGC selectivity criteria to be considered a BRPF specific probe. That is:

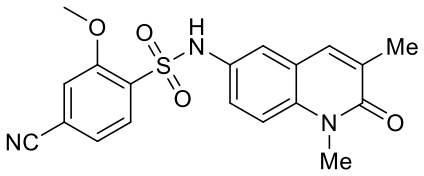
1. < 100 nM in potency in a biophysical assay.
2. 30 fold selective for the BRPF BRDs with respect to other class IV BRDs.
3. 100 fold selective over non-class IV BRDs.

BRD	K_D (nM)	Selectivity *vs* BRPF1 (× fold)	BRD	K_D (nM)	Selectivity *vs* BRPF1 (× fold)
ATAD2A	>50,000	>12,500	**BRD3(2)**	>50,000	>12,500
ATAD2B	>50,000	>12,500	**BRD4(1)**	3,300	825
BAZ2A	>50,000	>12,500	**BRD4(1,2)**	15,000	3750
BAZ2B	9,400	2350	**BRD4(2)**	>50,000	>12,500
BRD1	46	11.5	**BRD4(full-length)**	4,900	1225
BRD2(1)	5,900	1475	**BRD7**	100	25
BRD2(1,2)	12,000	3000	**BRD8(1)**	>50,000	>12,500
BRD2(2)	>50,000	>12,500	**BRD8(2)**	>50,000	>12,500
BRD3(1)	7,200	1800	**BRD9**	160	40

BRD3(1,2)	12,000	3000	**BRDT(1)**	4,800	1200
BRDT(1,2)	>50,000	>12,500	**PBRM1(5)**	11,000	2750
BRDT(2)	>50,000	>12,500	**PCAF**	4,200	1050
BRPF1	4	1	**SMARCA2**	>50,000	>12,500
BRPF3	110	27.5	**SMARCA4**	41,000	10250
CECR2	12,000	3000	**TAF1(2)**	4,900	1225
CREBBP	13,000	3250	**TAF1L(2)**	5,500	1375
EP300	15,000	3750	**TRIM24(Bromo)**	1,600	400
FALZ	2,300	575	**TRIM24(PHD,Bromo)**	5,600	1400
GCN5L2	4,100	1025	**TRIM33(PHD,Bromo**	>50,000	>12,500
PBRM1(2)	>50,000	>12,500	**WDR9**	>50,000	>12,500

Table 25. Bromonome K_D panel for **NI-57** . K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is presented as the mean of 2 replicates. Red = Class IV BRD.

However, as the DiscoverX K_D's had overestimated potency and selectivity for previous compounds, it was decided to determine K_D's by ITC in order to corroborate the data.



MW 383
tPSA 100 Å^2
ClogP 2.08

BRD	ΔT_m (°C)[a]	Dx K_D (nM)[b]	Dx K_D Selectivity *vs* BRPF1	ITC K_D (nM)[c]	ITC K_D Selectivity *vs* BRPF1
BRPF1	**10.7**	**4**	**-**	**31**	**-**
BRD1	**5.6**	**46**	**11.5**	**108**	**3.5**
BRPF3	**5.3**	**110**	**27.5**	**408**	**13**
BRD9	**3.1**	**160**	**40**	**997**	**32**

Table 26. Biochemical and biophysical evaluation of **NI-57** . a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates. c. ITC carried out by Cynthia Tallant at the SGC, n = 1

Changing the assay from a competition K_D to an ITC K_D lowered potency against all bromodomains. As for previous compounds the BRPF1 potency was most affected, dropping ~8 fold, similar to the drop seen for **124**. The decreases in potency for the other BRPF members, BRD1 and BRPF3, were both between 3 and 4, whilst BRD9 was in between at 5.5. Nonetheless, despite the large drop in

potency affecting compound selectivity, **NI-57** was still able to meet the probe criteria outlined by the SGC.

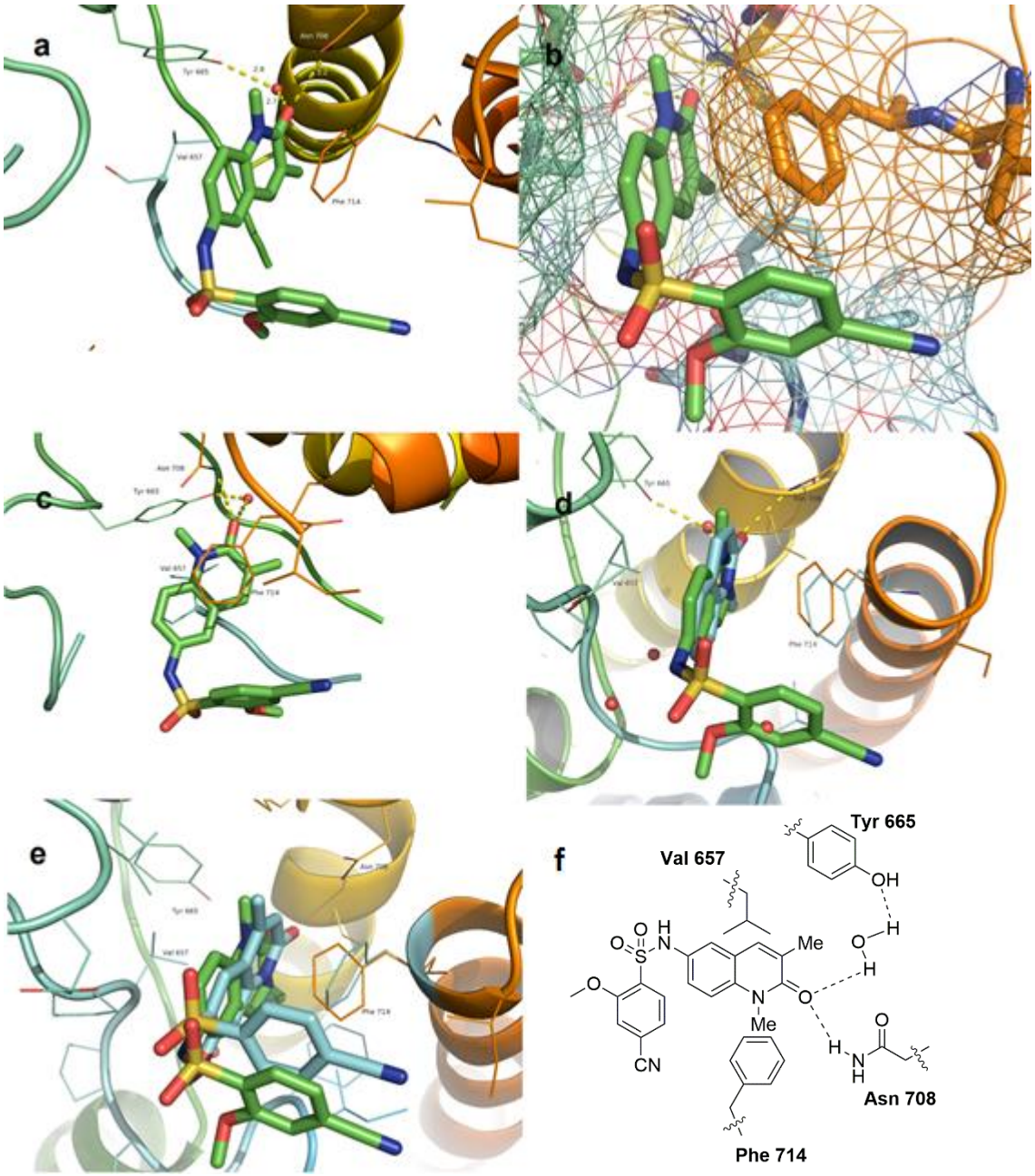


Figure 47. Cocrystal structure of **NI-57** with BRPF1 (2.10 Å) . Data obtained by Cynthia Tallant at the SGC. Diagram created in PyMOL. a. **NI-57** in BRPF1 highlighting the binding interactions between the KAc mimetic with N708 and W665 b. Surface of BRPF1 with **NI-57** bound c. **NI-57** bound to BRPF1 looking down F714. d. Superimposed crystal structures of **NI-57** and 1-methylquinolin-2(1*H*)-one (**14**- light blue) showing same binding mode, but 'tilting' further forward of F714 in **14** structure (light blue) e. Superimposed crystal structures of **NI-57** and **124** (light blue) showing how the quinolinone ring has flipped.d. 2 dimensional representation of the interactions between **NI-57** and the BRPF1 binding pocket.

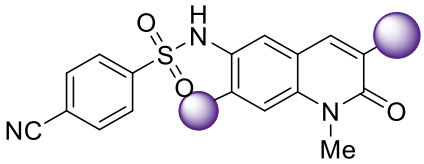
Having obtained a cocrystal structure of **NI-57** with BRPF1, the first thing of note was that the interactions between the acetyl-lysine mimetic and the BRPF1 binding pocket were maintained from **124** to **NI-57**. That is, the quinolinone carbonyl interacts directly with Asn 708 and *via* a water mediated hydrogen bond network with Tyr 665 (Figure 47a).

The first difference between the 2 crystal structures is highlighted in Figure 47b and c where, unlike **124** where Phe 714 forms a face-face π-stacking interaction with the quinolinone A-ring the aromatic ring, Phe 714 for **NI-57** is now tilted forward in a similar manner to that in the cocrystal structure of fragment **14** with BRPF1 (Figure 47d). Moreover the postulated T-shaped π-stacking interaction for **124** is no longer possible for **NI-57**.

Of particular note, is the overlayed cocrystal structures of **NI-57** and **124,** where it can be seen that the quinolinone ring has 'flipped', the *N*-alkyl group is now occupying the pocket formerly occupied by the 4-methyl group of **124.** Consequently, the **NI-57** sulfonamide aryl ring does not occupy the same area of the WPF shelf as **124**, bending in 'lower' than its **124** counterpart.

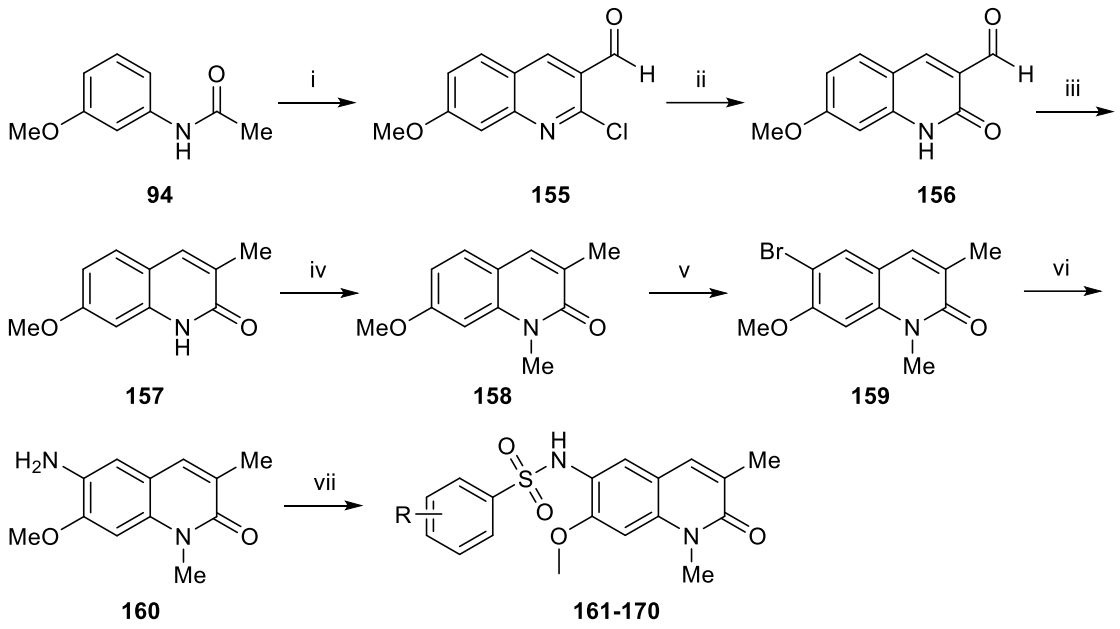
The cocrystal structure does not indicate why addition of the methyl ether to the sulfonamide aryl ring imparts greater potency and selectivity. There was no indication of an intramolecular hydrogen bond between the sulfonamide N*H* and the ether oxygen, or the T-shaped π-stacking which might have benefitted from the increased electron density of the aryl ring. Moreover, the methyl group does not appear to be binding in any hydrophobic pockets.

4.2.11 3-Me,7-OMe Substituted Quinolin-2(1*H*)-ones



On addition of a methyl ether at the 7 position of the quinolinone ring previous compounds had increased in both potency and selectivity. Inspection of the crystal structure (*vide supra*) showed that it was possible that the ethereal oxygen would be able to form an intramolecular hydrogen bond similar to that hypothesised to be responsible for the selectivity of compound **102**.

Thus 3-methyl-7-methoxy-6-sulfonamidequinolinones were synthesised according to scheme 26, in the hope of increasing the selectivity of **NI-57** against BRD7 and BRD9.



Scheme 26. Reagents and Conditions: i) $POCl_3$, DMF, 80 °C, 71%; ii) HCl (6.0 M), reflux, 64%; iii) Et_3SiH, CF_3COOH; 57%; iv) NaH, MeI, DMF, 81%; v) NBS, DMF, 73%; vi) cat. Cu_2O, NH_4OH (28–30% NH_3), NMP, μw, 110 °C; vii) RSO_2Cl, Pyridine, DMF.

Compound	R Group	Yield (%)	ΔT_m against BRPF1 (°C)[a]	K_D against BRPF1 (nM)[b]	ClogP
161	H	57	9.4	17	2.53
162	4-CN	49	8.8	15	2.47
163	2-Me, 4-CN	37	9.9	n.d.	2.97
164	2-OMe, 4-CN	37	12.7	12	2.07
165	2-OEt, 4-CN	22	9.5	n.d.	2.61
166	2-OMe	51	11.0	n.d.	2.07
167	2-OCF_3	49	n.d.	48	3.29
168	4-NO_2	66	6.6	n.d.	2.72
169	4-Me	60	7.7	n.d.	3.03
170	4-NH_2	75[c]	3.8	n.d.	1.65

Table 27. Biochemical and biophysical evaluation of compounds **161-170**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest

concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates. c. prepared by catalytic hydrogenation of compound **168.**

Reaction of compound **94** in a double Vilsmeier type reaction afforded 2-chloro-7-methoxyquinoline-3-carbaldehyde (**155**) in reasonable yield. [94] Hydrolysis of the 2-chloroquinoline in 3 M HCl provided the 3-formylquinolinone (**156**), [95] which was reduced with triethylsilane in TFA. Methylation, bromination, amination and coupling with the sulfonyl chloride were carried out as for previous reaction schemes. As for **105** the selectivity of the bromination step was confirmed by the presence of two singlets in the ^{1}H NMR spectrum, where the alternative 8-bromo isomer would have shown 2 doublets.

ID	BRPF1B	TIF1A	BRD4	BRD9	CECR2	CREBBP
161	9.4	0.8	2.6	4.2	-0.5	1.5
162	8.8	0.5	1.1	3.5	0.0	1.3
163	9.9	1.0	1.0	4.4	0.6	2.1
164	12.7	n.d.	n.d.	n.d.	n.d.	n.d.
165	9.5	n.d.	n.d.	5.3	0.1	n.d.
166	11.0	n.d.	n.d.	6.3	0.4	n.d.
167	Interferes with assay					
168	6.6	n.d.	n.d.	2.9	0.2	n.d.
169	7.7	n.d.	n.d.	2.8	0.0	n.d.
170	3.8	n.d.	n.d.	5.7	-3.5	n.d.

Table 28. ΔT_m results from sulfonamide investigation **161-170** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

The SAR generated for this core was consistent with that from previous series. Investigation of different groups at the 4-position of the sulfonamide ring suggested that a nitrile was the most favourable. Electron donating substituents (4-Me – **169** and 4-NH_2 –**170)** proved less potent. Indeed, compound **170** with a 4-NH_2 group was also inversely selective against BRD9 by TS. Decreasing electron donating ability relative to the nitrile was similarly ineffective, with 4-NO_2 (**168**) recording a T_m of 6.6 °C.

Addition of electron donating groups at the 2 position again appeared favourable, with all 2-position substituents recording appreciable thermal shifts. The data does imply that extension of the alkyl group from a methyl to an ethyl on the 2 position ether (**164** R = Me BRPF1 ΔT_m: 12.73 °C, **165** R = Et BRPF1 ΔT_m: 9.5 °C) is not beneficial, but there is insufficient data to confirm this hypothesis.

Following on from the promising T_m and K_D data sulfonamide **164** was assayed against the available bromonome by TS.

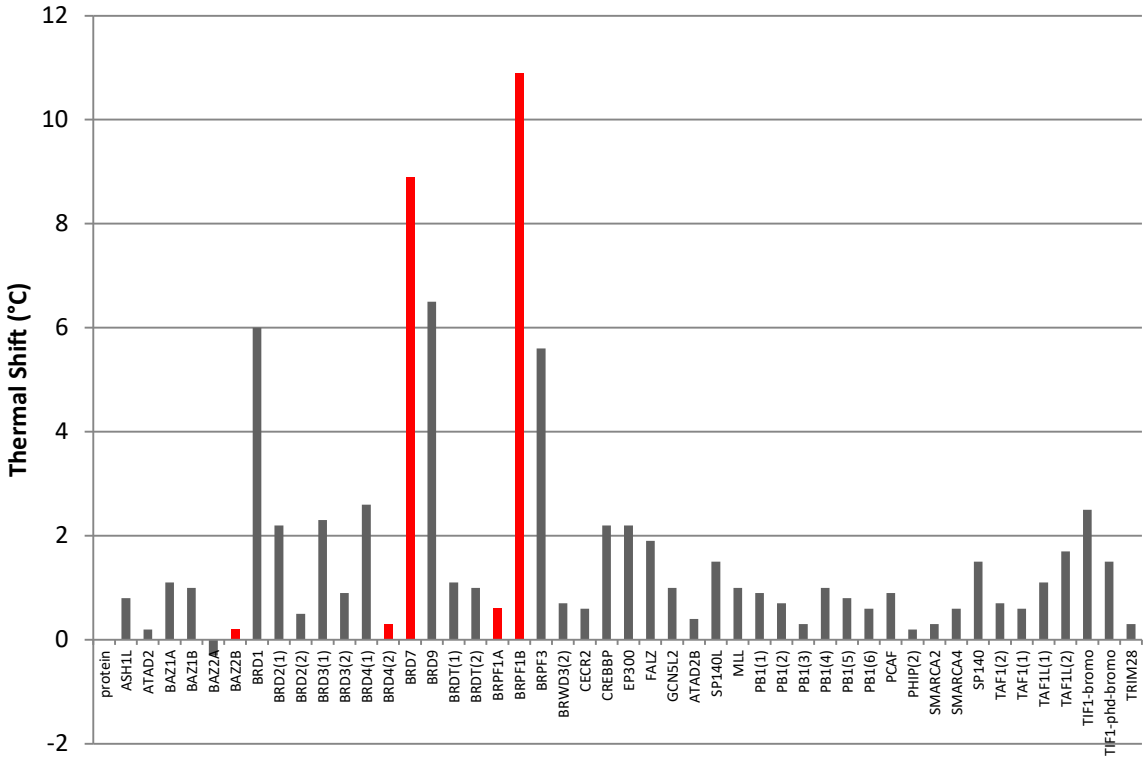


Figure 48. Graph showing bromonome screening of compound **164** by TS. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 3. Red = Class IV BRD

Results from the bromonome assay (Figure 48) indicated that, in contrast to previous compounds, addition of the methyl ether at the 7 position was counterproductive at improving compound selectivity. Selectivity was especially poor against key selectivity target, the class II BRDs (BRD2: 2.2 °C, BRD3: 2.3 °C and BRD4: 2.6 °C). Inspection of the cocrystal structures of **124** and **NI-57** suggests that if the sulfonamide orientates itself in such a manner as to form an internal hydrogen bond the inhibitor core will have to 'flip' again. As the potency against the class IV BRDs has been retained, but the non-class IV BRD potency has increased, the methoxy group promoted ring flip orientates the sulfonamide in a position more favourable for binding to other BRDs (Figure 49).

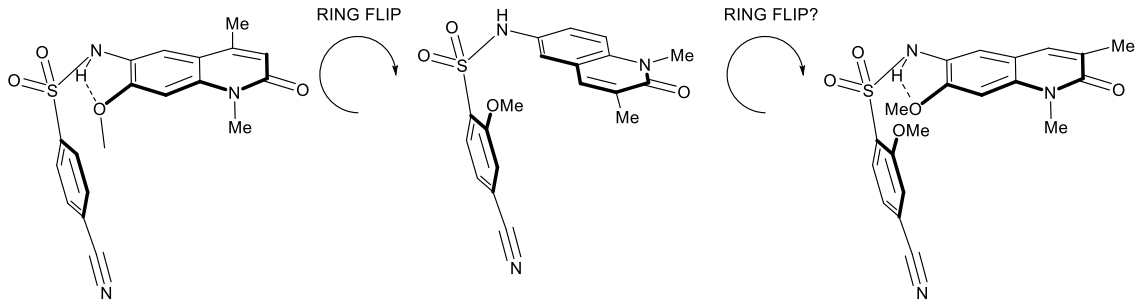
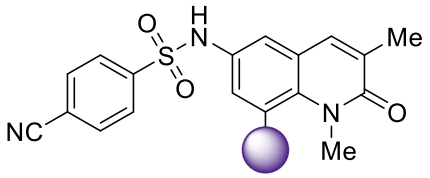


Figure 49. Binding mode of compound **124** on the left showing how the quinolinone ring 'flips' to the binding mode of **NI-57** in the middle. The hypothesised ring flip of compound **164** back into the original orientation as a consequence of the internal hydrogen bond is shown on the right

4.3 Inactive control

Having successfully synthesised **NI-57**, a BRPF selective chemical probe, and **NI-42** a tool compound for the class IV BRDs, we next turned our efforts towards the synthesis of inactive control compounds to help validate any biological results obtained. Several areas of the inhibitor were modified in these efforts

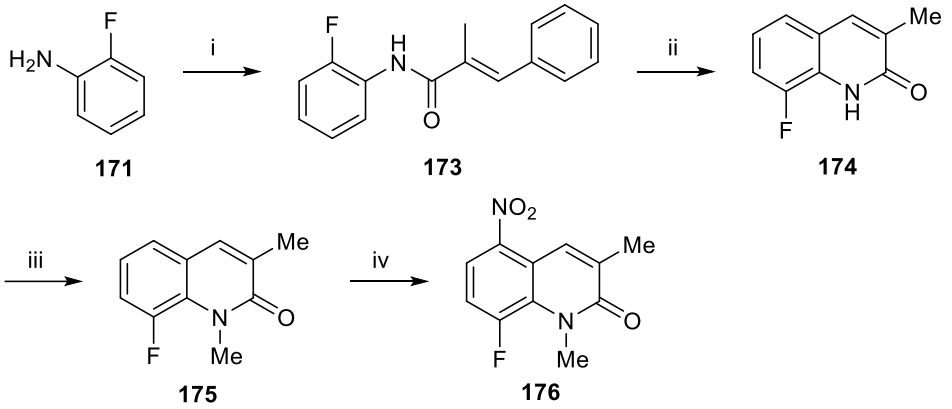
4.3.1 8-Fluoro Substituted Quinolin-2(1*H*)-ones



Initial attempts at producing an inactive analogue focussed on modification of the 8 position. It was envisaged that substitution of this site could interact with the 1 position alkyl group potentially causing the unfavourable torsion in the quinolinone ring, thus inhibiting binding. [96] Alternatively introduction, of electron withdrawing modifications could interact unfavourably with the proximal acetyl lysine mimetic, as had been observed for previous compounds.

Methylation of the unalkylated N*H* quinolinones was extremely low yielding, as a consequence of the peri-interactions between the 8 substituent and the approaching nucleophile. As a consequence of the steric clash almost exclusively *O*-alkylated product was isolated (or hydrolysed on silica).

In order to assess whether the 8 position substitution was tolerated, the sterically undemanding F was chosen as a starting point.



Scheme 27. Reagents and Conditions: i) α-methylcinnamoyl chloride (**172**), K_2CO_3, Acetone, H_2O, 88%; ii) $AlCl_3$, chlorobenzene, 110 °C, 84%; iii) NaH, MeI, DMF, 38%; iv) HNO_3, H_2SO_4, 0-5 °C, 71%

Reaction of 2-fluoroaniline (**171**) with α-methylcinnamoyl chloride (**172**) provided the appropriate cinnamide (**173**) which was cyclised in excellent yield using the previous employed conditions. Methylation afforded the required 8-fluoro-1,3-dimethylquinline-(1*H*)2-one (**175**) in good yield. Surprisingly nitration of this compound appeared to provide almost exclusively the unwanted 5-nitro regioisomer. This was unexpected as whilst both the fluoro and the amide are *ortho/para*

directing substituents the amide is usually the more powerful of the 2 (σ_{para} F: 0.06, NHCOMe: -0.15). [68] The 5-NO_2 assignment was based upon the following data:

1. The C_6H-C_7H coupling of 8.9 Hz is of a magnitude consistent with protons *ortho* to one another
2. The C_7H-C*F* and C_6H-C*F* coupling constants of 14 and 3.8 Hz respectively are consistent is consistent with substituents *ortho* and *meta* to a fluorine atom
3. Although not conclusive no proton interaction was observed in a NOESY between C_4H and a possible C_5H (Figure 50)

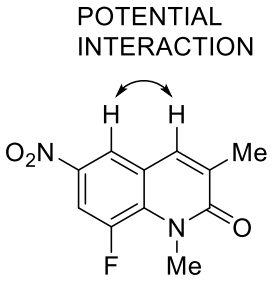
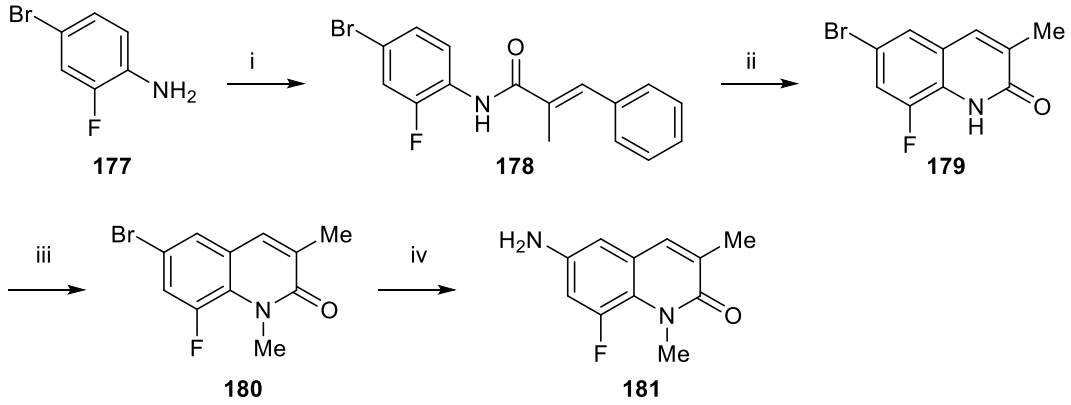


Figure 50. potential NOESY interaction between 4 and 5 position hydrogens

This was promising with respect to the hypothesis that the 8 substituent might cause ring torsion, as the reduced orbital overlap of the *para* directing amide might be responsible for the lack of desired 6-position nitration.

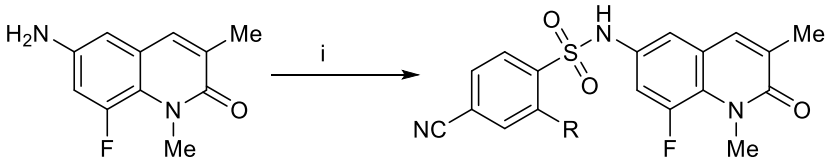
Thus, in order to be sure of synthesising a compound with the correct regiochemistry the route was repeated, but starting with 2-fluoro-4-bromoanline, which could eventually be subjected to standard amination conditions.



Scheme 28. Reagents and Conditions: i) alphamethylcinnamoyl chloride, K_2CO_3, Acetone, H_2O, 98%; ii) $AlCl_3$, chlorobenzene, 110 °C, 17%; ii) NaH, MeI, DMF, 25%; iv) cat. Cu_2O, NH_4OH (28–30% NH_3), NMP, μw, 110 °C

Standard amide formation conditions provided the appropriate cinnamide (**178**), although for this analogue issues were encountered on Friedel-Crafts cyclisation. TLC indicated substantial quantities of debrominated quinolinone, 8-fluoro-3-methylquinolin-2(1H)-one (**174**), in the supernatent. This was unexpected, as literature cyclization of N-(4-bromophenyl)cinnamamide

proceeded in 55% yield and cyclisation of the *des*-bromo cinnamide (**173**) was near quantitative. Despite these issues sufficient quantities of **180** were isolated to allow for methylation, amination and coupling to provide sulfonamides **182** and **183** for biological evaluation.



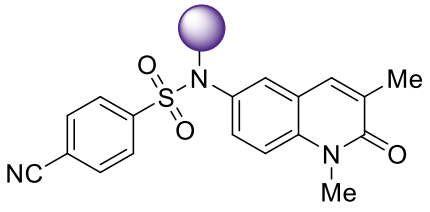
Scheme 29. Reagents and Conditions: i) RSO_2Cl, DMF, pyridine

Compound	R Group	Yield(%)*	ΔT_m against BRPF1 (°C)[a]	K_D against BRPF1 (nM)[b]	ClogP
182	H	21	7.1	n.d.	2.65
183	OMe	13	6.8	12	2.26

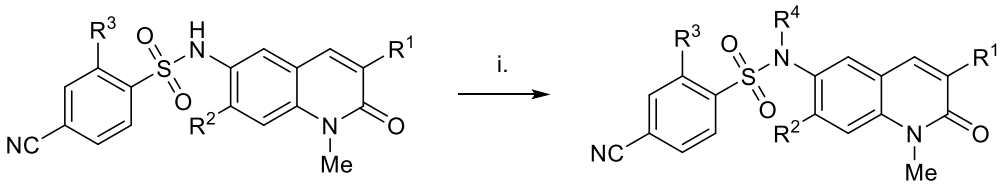
Table 29. Biochemical and biophysical evaluation of compounds **182** and **183**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates. * Yield over 2 steps from **180**.

The biological evaluation for these 2 compounds produced conflicting data. Thermal shift suggested that these compounds had significant drops in potency, however the K_D determined for compound **183** suggested that the drop in potency (~4 fold) was compariately modest. Whilst it is likely that the compounds had dropped somewhat in potency introduction of a fluorine at the 8 position was sufficiently tolerated to be unproductive for the synthesis of an inactive analogue.

4.3.2 Sulfonamide *N* – alkylation



Alkylation of the sulfonamide nitrogen was carried out by simple deprotonation with sodium hydride and subsequent reaction with the appropriate alkyl iodide in DMF



Scheme 29. Reagents and Conditions: i) NaH, R^4I, DMF

Compound	R^1	R^2	R^3	R^4	Yield	ΔT_m against BRPF1 (°C)[a]	K_D against BRPF1 (nM)[b]	cLogP
184	**H**	**H**	**H**	**Me**	**63**	**1.8**	**910**	**1.61**
185	**H**	**OMe**	**H**	**Me**	**63**	**-**	**1,000**	**1.58**
186	**Me**	**H**	**OMe**	**Me**	**62**	**n.d.**	**110**	**1.92**
187	**Me**	**H**	**OMe**	**Et**	**82**	**n.d.**	**110**	**2.45**
188	**Me**	**H**	**OMe**	**iPr**	**49**	**n.d.**	**160**	**2.76**

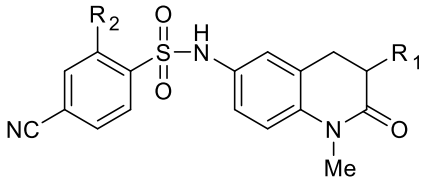
Table 30. Biochemical and biophysical evaluation of compounds **184-188**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

Methylation was first carried out on compound **23** and caused a ~20 fold decrease in compound potency (**23** BRPF1 K_D: 43 nM, **184** BRPF1 K_D: 910 nM). Similar drops in potency were observed for methylation of compounds **102** and **NI-57.** Clearly, whilst the sulfonamide N*H* involved is favourable, it is not vital to compound binding.

It was hoped that introducing a larger alkyl group would introduce a steric clash which would prevent the sulfonamide adopting the required orientation to bend into the WPF shelf. Thus **NI-57** was ethylated (**187**) and isopropylated (**188**) however the drops in potency were similar to those observed for compound methylation.

4.3.3 1-methyl-3,4-dihydroquinolin-2(1*H*)-one Core

Early SAR generated by the SGC suggested that the BRPF inhibition was reduced by reduction of the quinolinone A ring to form a 6-amino-1-methyl-3,4-dihydroquinolin-2(1*H*)-one core. Compounds synthesised early in the project (Dr Elliott Bayle) confirmed this, so a partially reduced **NI-57** was synthesised to investigate the effect this might have on BRPF inhibition

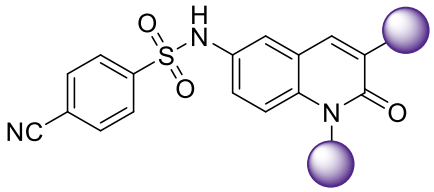


ID	R_1	R_2	ΔT_m against BRPF1 (°C)
189	H	H	3.3
190	Me	OMe	6.0

Table 31. Compounds synthesised by Dr. Elliott Bayle by hydrogenation. Biochemical and biophysical evaluation of compounds **189** and **190**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1

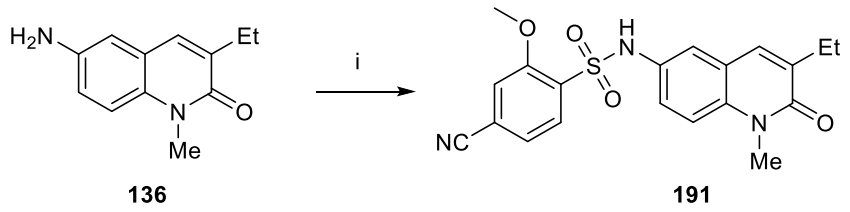
Whilst the effect of reducing the quinolinone A-ring double bond appeared to have a greater effect for **NI-57** on potency than for the earlier compound **23**, the reduction was not large enough to afford the desired inactive analogue.

4.3.4 1,3-Alkyl Variation



As it had been previously shown that introducing larger and longer alkyl chains at the 1 and especially the 3 positions of the quinolinone ring was detrimental to potency, it was next decided to investigate bigger alkyl groups at both positions, to probe the steric demands in proximity to the KAc binding site.

As the 3 position variation had led to a greater decrease in potency (**NI-42** to **137** potency dropped 23 fold) it was decided to synthesis the 3 Et analogue of **NI-57,** in order to determine whether the decrease in potency was mimicked for this sulfonamide.



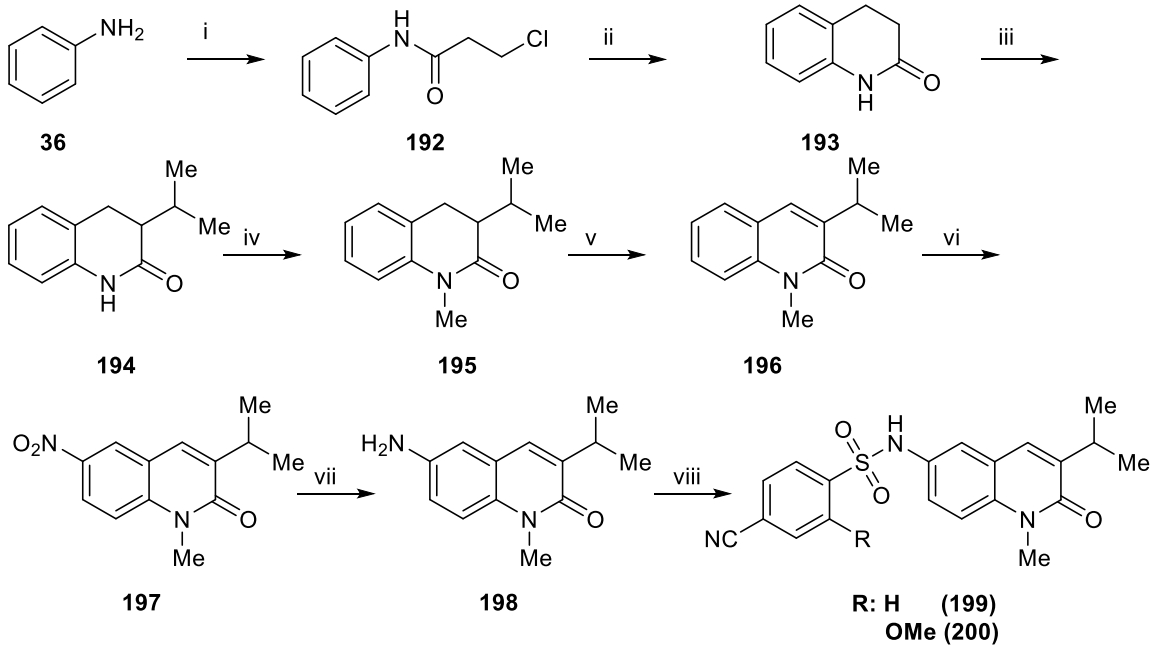
Scheme 29. Reagents and Conditions: i) RSO_2Cl, DMF, pyridine, 12% over 2 steps from **136**

ID	ΔT_m against BRPF1 (°C)	K_D against BRPF1 (nM)	cLogP
191	**6.9**	**120**	**2.61**

Table 32a. Biochemical and biophysical evaluation of compound **191**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1

Pleasingly the observed 30 fold decrease in affinity for BRPF1 was similar to that recorded for **NI-42** to **137**.

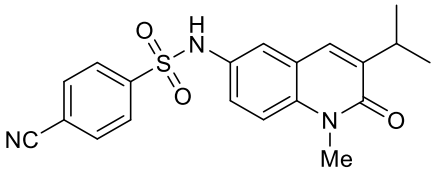
As increasing the size of the 3-alkyl group from Me to Et had proved effective at decreasing the potency we next extended the alkyl group to an isopropyl group. The sulfonamide was synthesised according to Scheme 30.



Scheme 30. Reagents and Conditions: i) 3-chloropropionyl chloride, K_2CO_3, acetone, 97%; ii) $AlCl_3$, chlorobenzene, 110 °C, 48%; iii) LDA, ^{i}PrBr, THF, -78 °C - RT, 48%; iv) NaH, MeI, DMF, 79%; v) DDQ, DCE, Reflux, 23%; vi) KNO_3, AcOH, 0 - 5 °C, 63%; vii) $SnCl_2{\cdot}2H_2O$, HCl, RT viii) DMF, pyridine, RSO_2Cl, **199** (34% over 2 steps) **200** (29% over 2 steps)

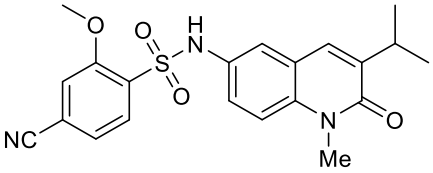
The *N*-(phenyl)-3-chloropropanamide (**192**) was synthesised as for compound **103** and cyclised by intramolecular Friedel-Crafts with $AlCl_3$ in chlorobenzene. *Alpha*-amide alkylation with LDA and isopropyl bromide provided 3-isopropyl-3,4-dihydroquinolin-2(1*H*)-one (**194**) in moderate yield.

Methylation, employing the standard protocol, was followed by oxidation by DDQ in DCE at reflux. Oxidation was incomplete after 2 days with 4 eq. of DDQ accounting for the low isolated yield of the desired quinolinone **196**. Initial nitration conditions employing sulfuric acid as the solvent with stoichiometric KNO_3 provided a variety of regioisomers. However, switching the solvents to the milder acetic acid provided the required 6-nitroisomer (**197**) in reasonable yield after crystallisation from ethanol. The quinolin-2(1*H*)-amide is *ortho* or *para* directing, thus nitration would be expected at either the 6 or 8 positions on the ring. The appearance in the NMR of a doublet with a *meta* coupling at 8.49 (J = 2.5 Hz) corresponding to C_5H, a doublet of doublets at 8.37 with *ortho* and *meta* couplings (J = 9.2, 2.5 Hz) corresponding to C_7H, and a doublet with *meta* coupling at 7.43 (J = 9.2 Hz) corresponding to C_8H is consistent with nitration at the desired 6 position. Reduction with $SnCl_2$ with concentrated HCl and subsequent coupling afforded the two sulfonamides (**199-200**) for testing against BRPF1 (Table 32).



199

MW 381
tPSA 90 Å^2
ClogP 3.39



200

MW 411
tPSA 100 Å^2
ClogP 3.01

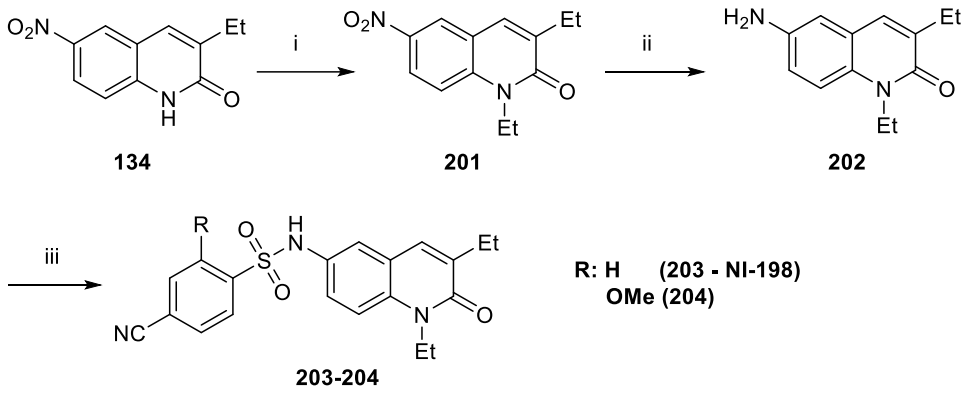
BRD	K_D (nM)	Decrease in Potency relative to NI-42 (× fold)
BRPF1	870	110
BRD1	14,000	291
BRPF3	16,000	61

BRD	K_D (nM)	Decrease in Potency relative to NI-57 (× fold)
BRPF1	170	43
BRD1	3,300	72
BRPF3	4,600	42

Table 33. Biophysical evaluation of compounds **199** and **200**. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

Altering the alkyl group at the 3-position for the larger isopropyl was more effective at impeding compound binding for the analogue of **NI-42** (**199**), than for the analogue of **NI-57** (**200**). Despite the significant drop in compound affinities for all three BRPF BRDs, even for compound **199** the difference was insufficient to be considered a true inactive control. Nonetheless, the results highlighted that further alteration of the alkyl groups in this area could prove fruitful for design of an inactive control. Therefore, it was decided to synthesise the 1,3 ethyl compound which would be

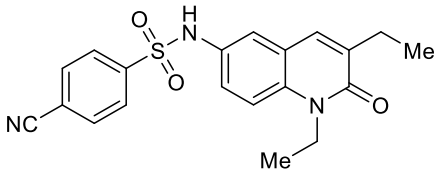
tractable from the previously synthesised 3-ethyl-6-nitroquinolin-2(1*H*)-one (**135**). The synthetic strategy utilised for synthesis of the desired sulfonamides is outlined in Scheme 31



Scheme 31. Reagents and Conditions: i) NaH, EtI, DMF, 65%*; ii) $SnCl_2$, HCl, 44%*; iii) RSO_2Cl, DMF, Pyridine, **203** (14% over 3 steps, **204** (20% over 3 steps) * indicates crude yield

Ethylation was carried out as for previous compounds, however unlike the *N*-methyl compound (**135**) $SnCl_2$ was employed as a reductant prior to sulfonamidation.

Ethylation of both the 1 and 3 positions of the quinolinone was successful in creating an inactive control for **NI-42**. Compound **203** (henceforth **NI-198**) has affinity reduced by > 1,000 fold against all 3 members of the BRPF family and the rest of the class IV BRDs. Further profiling against BRD4 and representative BRDs from the other BRD families established that **NI-198** showed minimal affinity for the other BRDs.



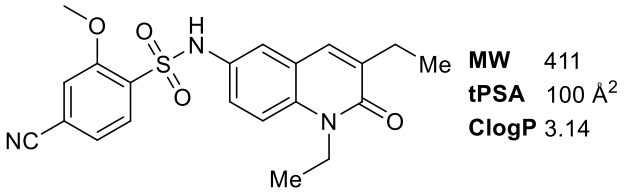
MW 381
tPSA 90 Å^2
ClogP 3.52

BRD	K_D (nM)	Decrease in Potency relative to NI-42 (× fold)	BRD	K_D (nM)	Decrease in Potency relative to NI-42 (× fold)
BRPF1	> 100,000	> 1,000	ATAD 2B	> 100,000	n.d.
BRD1	> 100,000	> 1,000	BRD4(1)	> 100,000	n.d.
BRPF3	> 100,000	> 1,000	CREBBP	> 100,000	n.d.
BRD7	> 100,000	> 1,000	PCAF	> 100,000	n.d.
BRD9	> 100,000	> 1,000	SMARCA2	77,000	n.d.
ATAD2A	> 100,000	n.d.	TRIM33	> 100,000	n.d.

Table 34. Biophysical evaluation of compound **NI-198**. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the

measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

Whilst the same motif was more effective than the 3-isopropyl compound at reducing compound affinity, the effect was not so drastic for 1,3 Et analogue of **NI-57**. Compound **204** was only 62.5 times less potent against BRPF1. This was surprising as it was hypothesised that the compounds would have identical binding modes, and would therefore be similarly affected by changes in such close proximity to the KAc mimetic.



BRD	K_D (nM)	Decrease in Potency relative to NI-57 (× fold)
BRPF1	250	62.5
BRD1	4,400	96
BRPF3	4,600	42

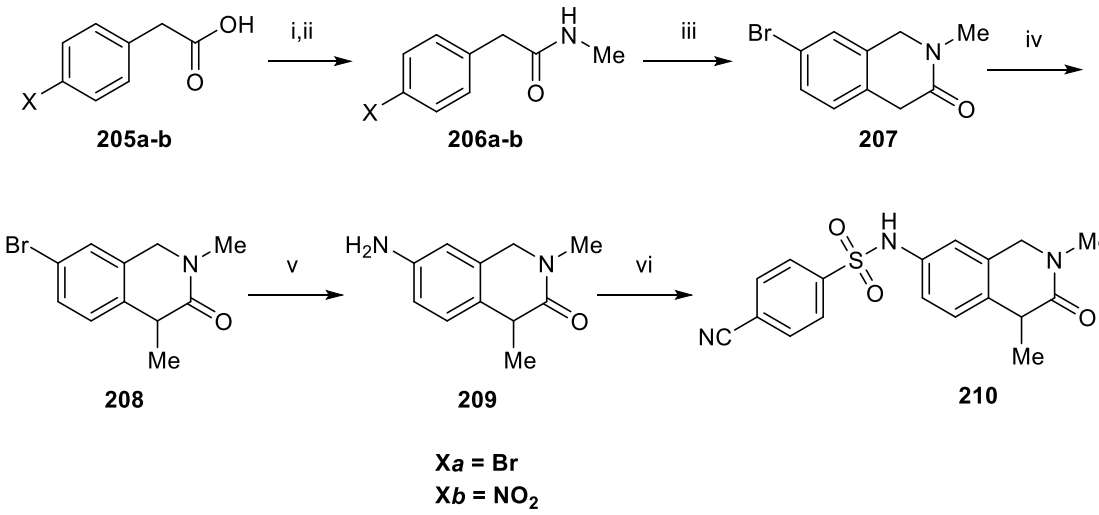
Table 35. Biophysical evaluation of compound **204**. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

4.4 Second Generation Inhibitors

We next focussed on generating BRPF inhibitors based on alternative cores, with the aim of designing inhibitors with different selectivity profiles.

4.4.1 2,4-dimethyl-1,4-dihydroisoquinolin-3(2*H*)-ones

As reduction of the quinolinone A ring did not appear to have a drastic effect on potency, the core was altered to the 7-amino-2,4-dimethyl-1,4-dihydroisoquinolin-3(2*H*)-one.



Scheme 32. Reagents and Conditions: i) SO_2Cl, DMF (cat), Toluene, 45 °C; ii) $MeNH_{2(aq)}$, 0 °C, a: 98%, b: 90%; iii) Paraformaldehyde, Eaton's reagent, 80 °C, a: 70%, b: 0%; iv) LDA (1M in THF), -78 °C *then* MeI -78 °C - RT, 41%; v) cat. Cu_2O, NH_4OH (28–30% NH_3), NMP μw, 110 °C; vi) RSO_2Cl, DMF, Pyridine, 16% over 2 steps

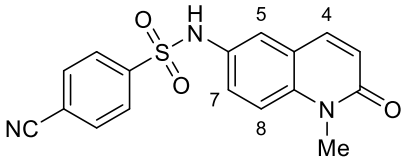
Synthesis of the required 7 substituted 2-methyl-1,4-dihydroisoquinolin-3(2*H*)-one was carried out as per the procedure of Ulysse *et al.* [97] Whilst the formation of the acid chloride and subsequent reaction with aqueous methylamine proceeded as described, for the strongly electron withdrawing nitro group the Pictet-Spengler type reaction failed to furnish any of the cyclised product. Thus the nitro group was exchanged for a bromine for which transformation into the *N*-methylamide and subsequent cyclisation were successful (**207**). This was followed by *alpha*-amide alkylation with LDA and iodomethane in modest yield. Amination *via* the standard procedure provided the crude amine which was coupled to give sulfonamide **210** for biological evaluation against BRPF1

Unfortunately, the sulfonamide **210** only inhibited BRPF1 with a ΔT_m of 3.61 °C and thus this series was not further investigated

4.4.2 Napthyridin-2(1*H*)-ones

Substitution of one of the carbon atoms in the quinolinone ring for a nitrogen, to make a napthyridinone, could alter the physicochemical properties of the final sulfonamides quite drastically depending which carbon on the ring was substituted (Table 35). The two properties most affected would be the pKa of the sulfonamide nitrogen, and the ClogP.

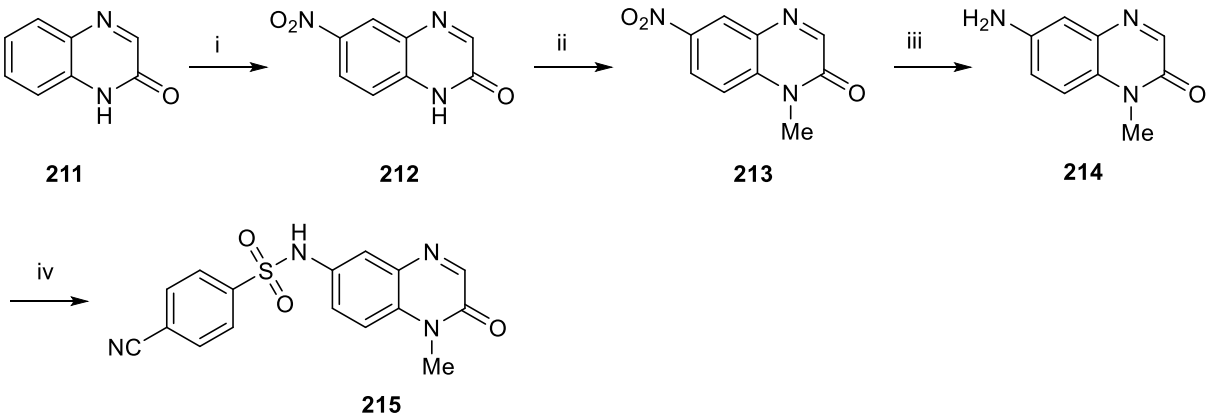
If the sulfonamide is *ortho* to the napthyridinone nitrogen it would result in a significantly more acidic sulfonamide proton. Moreover according to *in silico* predictions the ClogP would decrease by up to 1 log unit depending on where in the ring the nitrogen is substituted.



Position of Nitrogen	CLogP
-	1.96
4	1.31
5	1.23
7	1.02
8	1.02

Table 36. Position of nitrogen in the quinolone-1(2*H*)-one and the effect on CLogP

As the most synthetically tractable of the desired napthyridinones the 4 position was the first investigated



Scheme 33. Reagents and Conditions: i) HNO_3, H_2SO_4, 0-5 °C, 92%; ii) NaH, MeI, DMF, 78%; iii) $SnCl_2$, conc HCl; iv) RSO_2Cl, pyridine, DMF, 8% over 2 steps

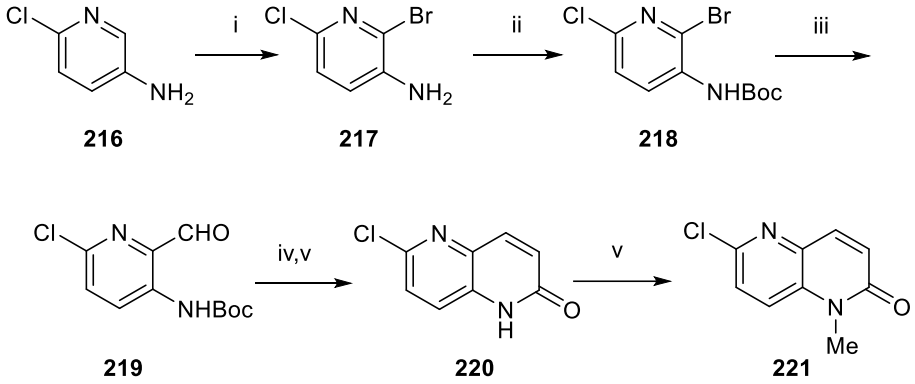
Nitration and subsequent methylation, reduction and coupling of 4-quinaxalinol (**214**) was carried out using standard conditions outlined in Scheme 33. Problems were encountered during the reduction step, as the strongly acidic conditions appeared to promote rapid amine degredation, but shortening of the reaction times lead to sufficient quantities being isolated for coupling.

ID	BAZ2A	BRD1	BRD4	BRD9	BRPF1B	BRPF3	CECR2	CREBBP	FALZ
215	0.0	-0.1	-0.2	1.6	2.4	0.6	0.0	-0.9	-0.8

Table 37. ΔT_m results for **215** (°C). Results obtained by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. Heat map is a scale from Green = low ΔT_m – low potency to Red = high ΔT_m - high potency.

The replacement of the carbon at the 4-position with a nitrogen resulted in a substantial drop in potency against BRPF1 by TS (**23** BRPF1 ΔT_m 5.2 °C). In addition, this drop in potency was not propagated throughout the class IV BRDs, as BRD9 potency had only dropped 0.65 °C. As a consequence the selectivity window was likely to be smaller for compound **215.**

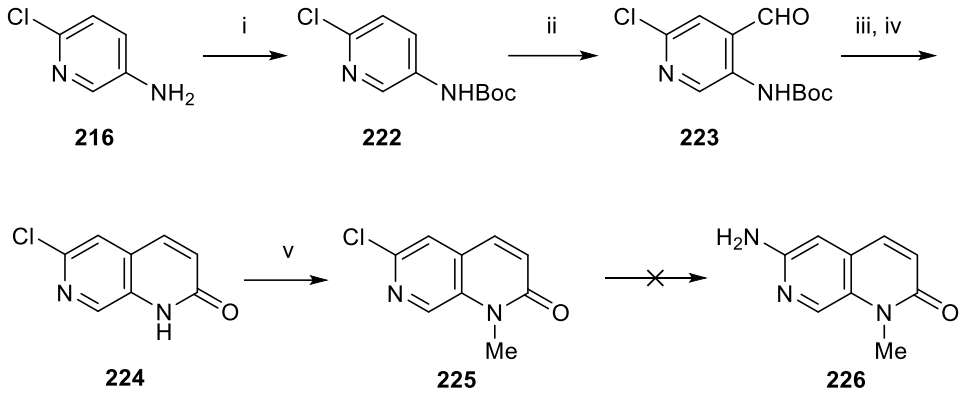
The next napthyridinones to be synthesised were those where the nitrogen replaced the carbon at the 5 and 7 positions respectively. Similar synthetic strategies were employed for both analogues.



Scheme 34. Reagents and Conditions: i) NBS, DMF, 82%; ii) KHMDS (1.0 M in Toluene), Boc_2O, Toluene, 69%; iii) MeLi (1.0 M in THF), BuLi (1.6 M in Hexanes), THF -78 °C *then* DMF -78°C to RT, 41%; iv) LDA (1.0 M in THF) -78 °C, *tert*-butylacetate *then* **218**, -78 °C to RT; v) HCl (3.0 M), 1,4 Dioxane, 100 °C, 38% over 2 steps; vi) NaH, MeI, DMF, 65%.

For the 5-position analogue, the literature procedures of Grongsaard *et al* and Turner *et al* were combined. [81,81, 98] Thus, 3-amino-6-chloropyridine (**216**) was first brominated in DMF in good yield, followed by installation of the *tert*-butylcarbonate group as an *ortho* directing metallating group. Deprotonation of the amide with MeLi was followed by lithium-halogen exchange with BuLi to provide an anion for reaction with the aldehyde equivalent DMF. The subsequent aldol reaction, cyclisation and methylation were identical to those in Scheme 13.

Synthesis of the 6-chloro-1-methyl-1,7-naphthyridin-2(1*H*)-one followed a similar route, with initial reaction of the amine with the *ortho* directing *tert*-butylcarbonate group. Without the bromine group to guide lithium halogen exchange at the 2 position of the ring the butyl lithium deprotonates the ring at the 4 position. Ensuing reactions followed the outline in Scheme 35.

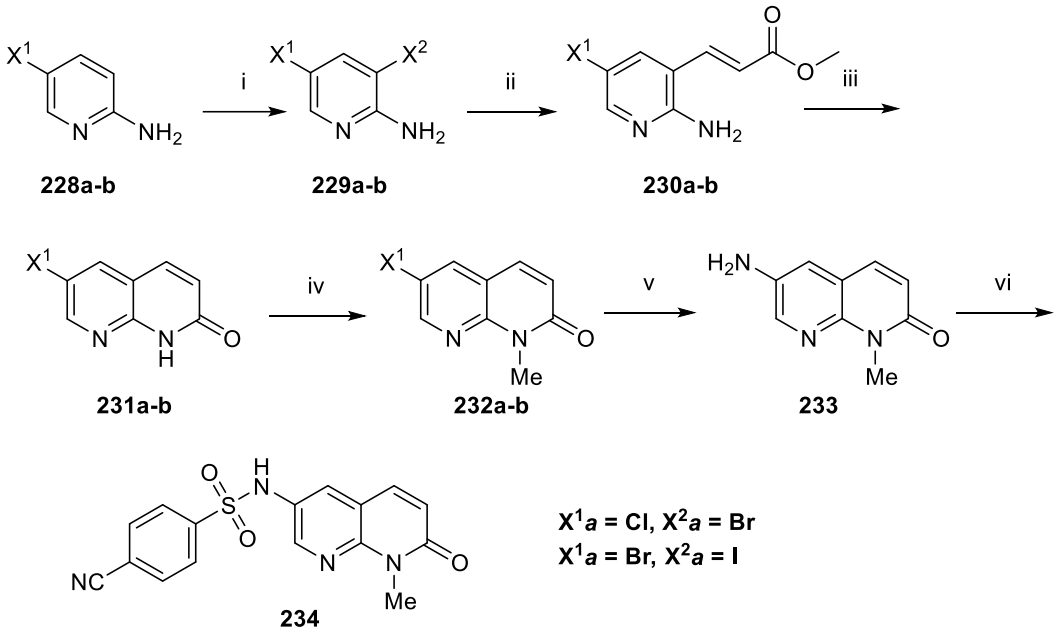


Scheme 35. Reagents and Conditions: i) Boc_2O, 1,4 Dioxane, 110 °C, 88%; ii) BuLi (1.6 M in Hexanes), TMEDA, THF -78 °C *then* DMF -78°C to RT, 37%; iv) LDA (1.0 M in THF) -78 °C, *tert*-butylacetate *then* **223**, -78 °C to RT; iv) HCl (3.0 M), 1,4 Dioxane, 100 °C, 50% over 2 steps; v) NaH, MeI, DMF, 80%.

With compounds **221** and **225** in hand, conditions were next investigated for aminoloysis of the 2-chloropyridine moiety. Both compounds **221** and **225** proved inert to reaction with aqueous and methanolic (2 M and 7 M) ammonia, even at elevated temperatures (130 °C for methanolic, 160 °C for aqueous). Employing the previously successful microwave conditions with catalytic Cu_2O was also ineffective.

Direct cross-coupling with 2-methoxy-4-cyanobenzenesulfonamide (**227**) was thus attempted. Unfortunately despite screening several catalysts and conditions the starting material also proved inert to coupling conditions.

Preparation of the 6-chloro-1-methyl-1,8-napthyridone (**232a**) followed an adaptation of literature procedure, involving initial bromination at the 2 position to provide compound **229a**. Subsequent Heck coupling with methyl acrylate, followed by cyclisation with methanolic sodium methoxide provided **231a** in good yield. Reaction with iodomethane yielded the required core for sulfonamidation. [99] Unfortunately, like the other 6-chloronapthyridones it proved inert to the coupling conditions attempted. Therefore the halogen was substituted for the more labile bromine, with a near identical synthetic strategy employed



Scheme 36. Reagents and Conditions: i) a. Br_2, $CHCl_3$, 62%; b. NIS, DMF, 47%; ii) methylacrylate, $Pd(OAc)_2$, NEt_3, $P(Ph)_3$, DMF a: 59%, b: 65%; iii) NaOMe, MeOH, reflux, a: 88%, b: 76%; iv) NaH, MeI, DMF, a: 71%, b: 81%; v) cat. Cu_2O, NH_4OH (28–30% NH_3), NMP μw, 110 °C; vi) RSO_2Cl, DMF, Pyridine, 13% over 2 steps.

Reaction of the 6-bromo-1-methyl-1,8-napthryidone (**232b**) using the standard microwave conditions proved successful and the crude amine was coupled to provide **233** for biological evaluation against BRPF1. Dissociation constant determination showed that **234** was an 870 nM inhibitor of BRPF1, approximately 18 times less potent than its quinolinone analogue (**23**)

The three 6-chloronapthyridones were also tested in an attempt to quantify the effect of the nitrogen on BRPF binding

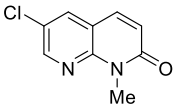
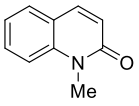
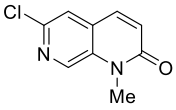
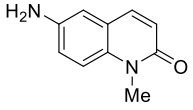
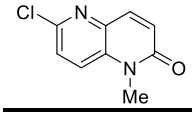
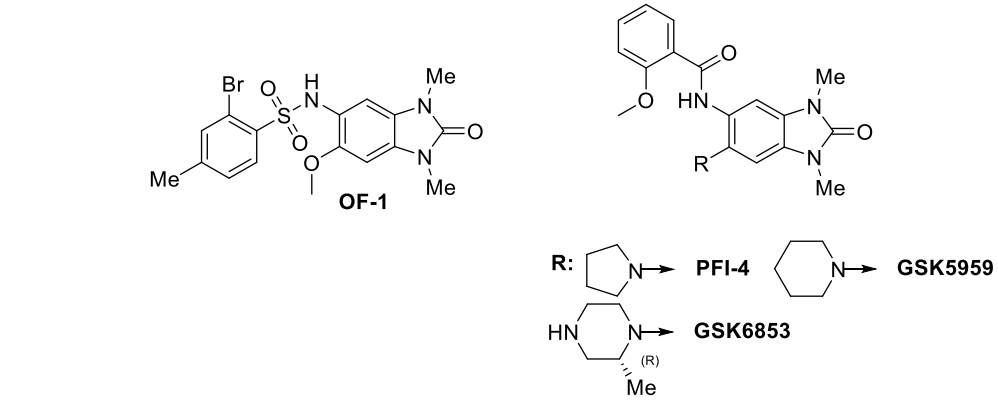
Fragment	K_D BRPF1 (nM)	CLogP	Fragment	K_D BRPF1 (nM)	CLogP
	> 100,000	1.02		4,800	1.30
	15,000	1.02		2,100	0.44
	20,000	1.23			

Table 38. Biophysical evaluation of compound napthyridone fragments. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

In general the addition of the nitrogen appears to have decrease binding affinity relative to the unaltered quinolinone ring with the 8 nitrogen being especially unfavourable. It was thus unsurprising that compound **234** recorded a K_D of 870 nM.

4.5 Hybrid inhibitors

During the course of our project a number of BRPF1 inhibitors were published in the literature, most notably the structurally similar BRPF1 specific inhibitors from Pfizer (**PFI-4)** and GSK (**GSK5959** and **GSK6853**) and the pan-BRPF inhibitor from the SGC (**OF-1**) (see section 3.4).



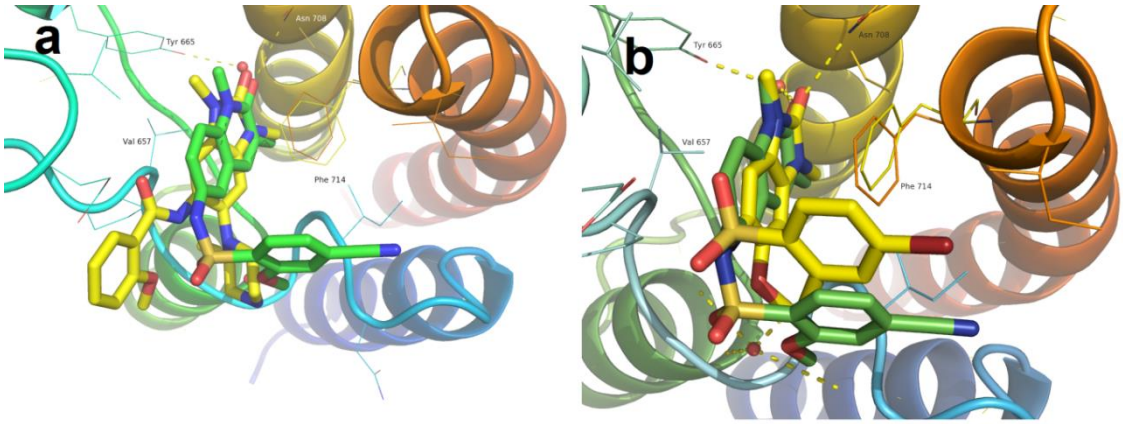


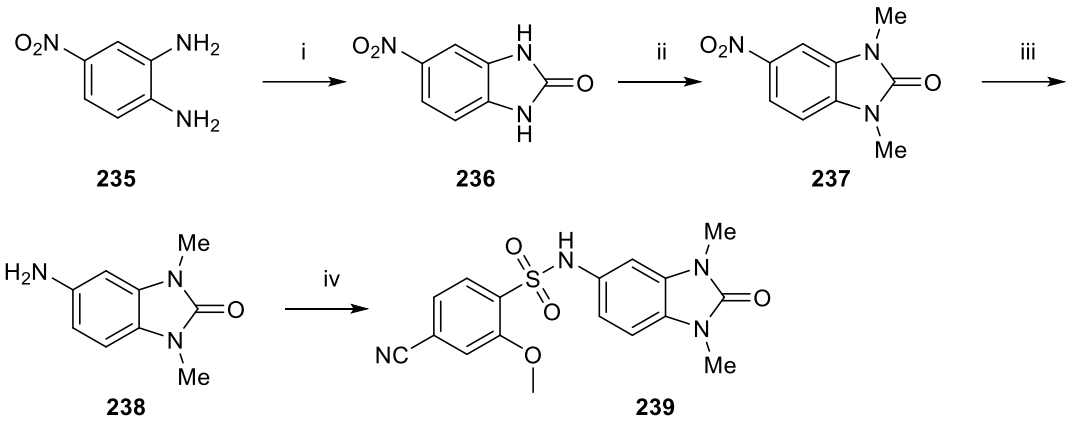
Figure 51. Overlayed cocrystal structures of BRPF1 with **NI-57** and published BRPF inhibitors. a. Overlay of **NI-57** and **GSK6853** crystal structures highlighting the identical binding interactions, but different binding modes (PDB ID: 5G4R) b. **NI-57** and **OF-1** highlighting similar binding modes in the WPF shelf and identical binding interactions (PDB ID: 5FG4)

Overlaying the crystal structures of the published inhibitors highlights that the acetyl lysine mimetics form identical binding interactions, but that as expected the amide from the Pfizer and GSK probes does not bend into the WPF shelf like the sulfonamides of **NI-57 a**nd **OF-1**. Of particular note is that the 2 cores, the benzo[d]imidazole-2-one and the quinolin-2(1*H*)-one cores appear to overlay well with the 6 position nitrogen occupying the same area of the protein for both cores.

4.5.1 1,3-Dihydro-2H-benzo[d]imidazol-2-one Acetyl Lysine Mimetic

The main disadvantages of the SGC pan BRPF inhibitor **OF-1** were that it had modest BRD4 inhibition, as well as very low solubility. It was thought that by hybridising the **OF-1** 1,3-Dihydro-2H-benzo[d]imidazol-2-one core with the sulfonyl chloride from **NI-57** it might be possible to create a more soluble and selective inhibitor

For synthetic tractability the unsubstituted benzo[d]imidazole-2-one was first synthesised according to Scheme 37.



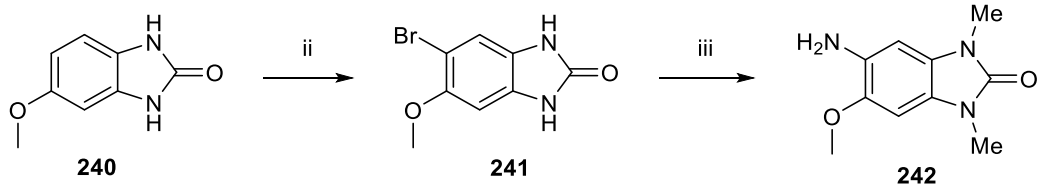
235 236 237 238 239

Scheme 37. Reagents and Conditions: i) 1,1'-Carbonyldiimidazole, DMF, 90%; ii) NaH, MeI, DMF, 46%; iii) 5% Pd/C, H_2, MeOH, 82%; iv) RSO_2Cl, pyridine, DMF, 55%.

Reaction of 1,2-Diamino-4-nitrobenzene (**235**) with 1,1'-carbonyldiimidazole in DMF gave compound **236** in good yield, [100] which was methylated under standard conditions. Reduction of the nitro group with palladium on carbon provided the amine (**238**) in excellent yield which was coupled using the previously employed procedure.

Sulfonamide **239** was evaluated by K_D which indicated a potency of 230 nM against BRPF1. Similarly to literature compounds based on the same core, sulfonamide **239** displayed very poor solubility as ~4 mg required heating and extensive sonication to dissolve in around 500 µL of DMSO.

Synthesis of the hybrid inhibitor containing the 5-OMe group was attempted according to Scheme 38.



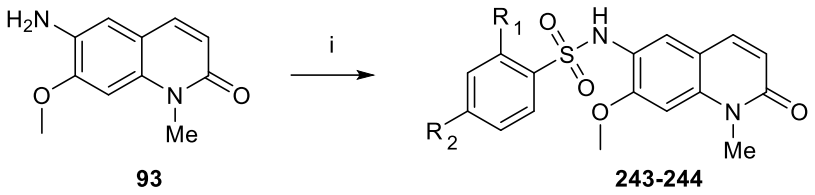
240 241 242

Scheme 38. Reagents and Conditions: i) NBS, DMF, 84%; ii) NaH, MeI, DMF, 98%.

Attempts at literature nitration of **240,** which could then be reduced and coupled, were unsuccessful in our hands, exclusively isolating the polynitrated compound. Bromination of compound **240** which could then be methylated and coupled, either *via* the amine or directly to the sulfonamide was thus carried out. Bromination and methylation proceeded smoothly, however attempts at direct coupling with the sulfonamide or transformation into the amine were unsuccessful. Later, GSK published a route to the 7OR-6NO_2-benzo[d]imidazole-2-one however, as the methoxy group was unlikely to drastically improve solubility, efforts to synthesise this hybrid were not pursued.

4.5.2 Quinolin-2(1*H*)-one Hybrids

The first hybrids synthesised were those that combined the 6-amino-7-methoxy-1-methylquinolin-2(1*H*)-one (**93**) core with the sulfonamides from **OF-1**, (2-methyl-4-bromobenzenesulfonyl chloride) and **NI-57** (2-methoxy-4-cyanobenzensulfonyl chloride-**148**).



93 **243-244**

Scheme 39. Reagents and Conditions: i) RSO_2Cl, DMF, Pyridine

Compound	R_1/R_2 Group	Yield*	ΔT_m against BRPF1 (°C)[a]	K_D against BRPF1 (nM)[b]	ClogP
243	R_1 - Me, R_2 - Br	22	6.3	n.d.	3.49
244	R_1 - OMe, R_2 - CN	18	n.d.	49	1.58
102	R_1 - H, R_2 - CN	21	5.6	52	1.96

Table 39. Biochemical and biophysical evaluation of compounds **243** and **244**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates. *Yield over 2 steps from **100**.

Hybridising with the **OF-1** sulfonamide, to give compound **243** yielded a sulfonamide which appeared to be more potent by TS, but wider screening with this assay indicated that the compound was significantly less selective (data not shown).

Efforts at improving the solubility and increasing the potency of the excellent selectivity seen previously for this core, by coupling to 2-methoxy-4-cyanobenzenesulfonyl chloride (**244**) proved ineffective, as there was no increase in potency and selectivity was largely similar.

4.5.3 PFI-4 Hybrids

We next turned towards amalgamating **PFI-4** and **NI-57**, by retaining the 1-methylquinolin-2(1*H*)-one core lysine mimetic, but substituting the sulfonamide for the 2-methoxybenzamide and installing the 7-pyrrolidine ring. It was envisaged that 2 analogues would need to be synthesised, as unlike the 1,3-dihydro-2H-benzo[d]imidazol-2-one core, the 1-methylquinolin-2(1*H*)-one is not C_2 symmetric (Figure 52).

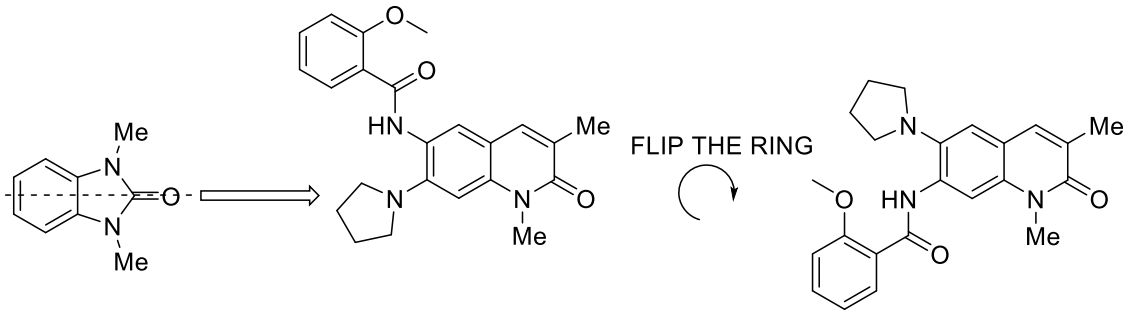
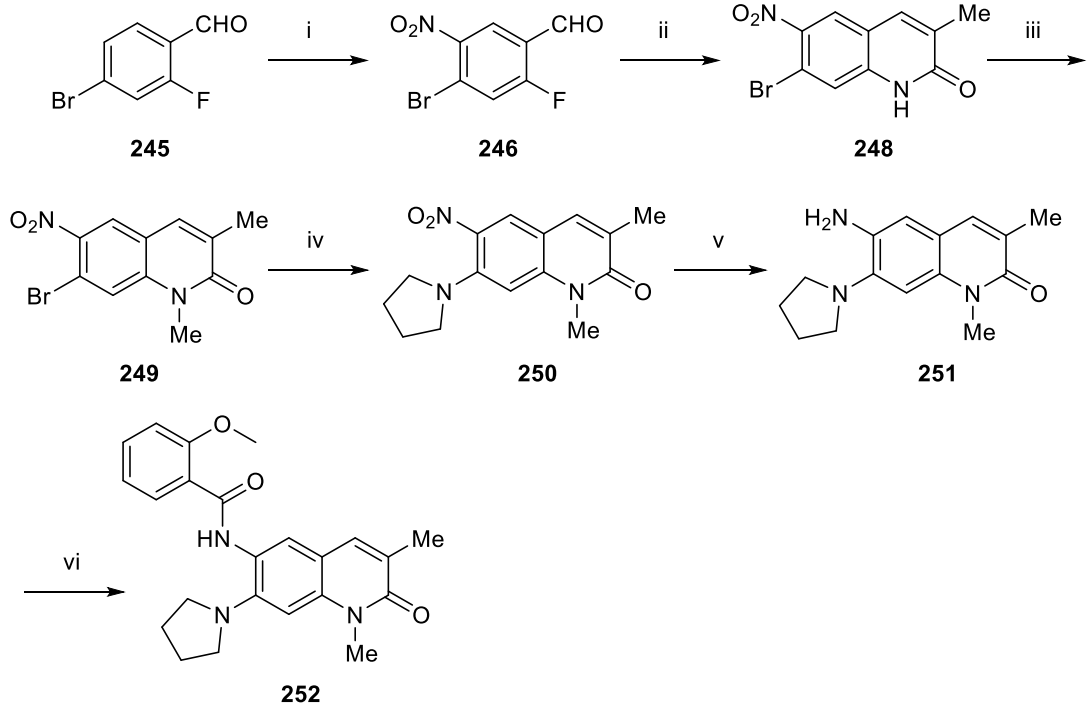


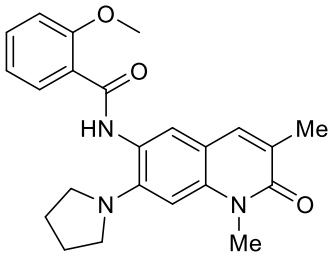
Figure 52. C2 Symmetry of the 1,3-Dihydro-2H-benzo[d]imidazol-2-one core and the 2 required regioisomers required to investigate the PFI-4 and NI-57 hybrid

The first analogue to be synthesised was the 6-amide *N*-(1,3-dimethyl-2-oxo-7-(pyrrolidin-1-yl)-1,2-dihydroquinolin-6-yl)-2-methoxybenzamide (**252**). The polysubstituted ring structure was efficiently accessed using the synthetic strategy outlined in Scheme 40.



Scheme 40. Reagents and Conditions: i) H_2SO_4, HNO_3, 0-5 °C, 89%; ii) $NaHSO_4$, 2-ethyl-4,4-dimethyl-4,5-dihydrooxazole (**247**), Xylene, NMP RT - 110 °C *then* 110 - 210 °C, 82%; iii) NaH, MeI, DMF, 78%; iv) pyrrolidine, CuI (cat), DMF, μw 100 °C, 65%; v) $SnCl_2$, conc. HCl, 91%; vi) RCOCl, pyridine, DMF, 36%.

Literature nitration of **245** yielded **246** in excellent yield, which was subjected to the cyclisation conditions previously employed in Scheme 22. [101] Methylation *via* standard conditions was followed by an Ullman/S_NAr replacement of the bromine with pyrrolidine. Reduction of the nitro group in moderate yield preceded coupling with 2-methoxybenzoyl chloride to give **252** for evaluation against BRPF1.

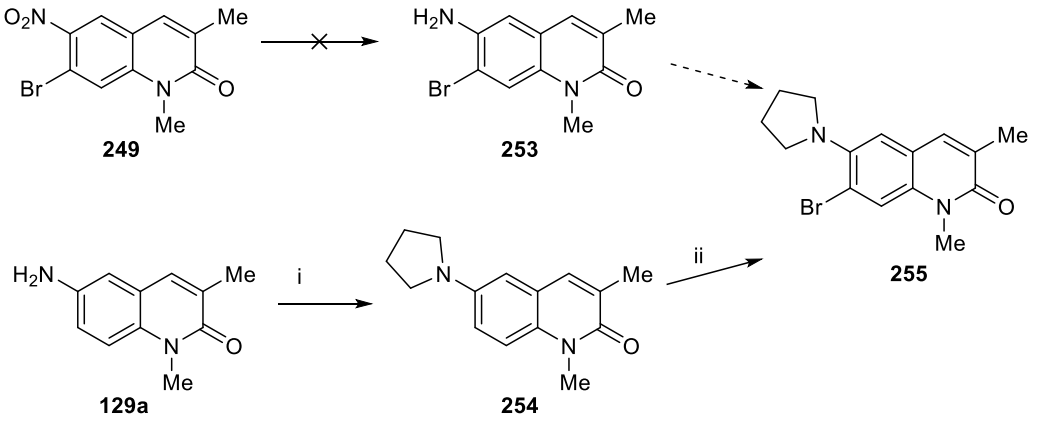


MW 391
tPSA 65 Å^2
ClogP 3.05

BRD	K_D (nM)	Selectivity *vs* BRPF1 (× fold)	BRD	K_D (nM)	Selectivity *vs* BRPF1 (× fold)
BRPF1	**220**	**-**	**BRD9**	**420**	**1.9**
BRD1	**2,600**	**12**	**BRD4**	**4,600**	**42**
BRPF3	**12,000**	**55**			

Table 40. Biochemical and biophysical evaluation of compound **252** a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

Given the low potency against BRPF1 and poor selectivity against other BRDs, amide **252** is clearly unable to adopt the correct binding orientation to achieve the profile observed for **PFI-4**. The inability of the amide to bind in the channel may be a result of the quinolinone ring flipping. Thus synthesis of the alternative regioisomer was attempted according to Scheme 41.



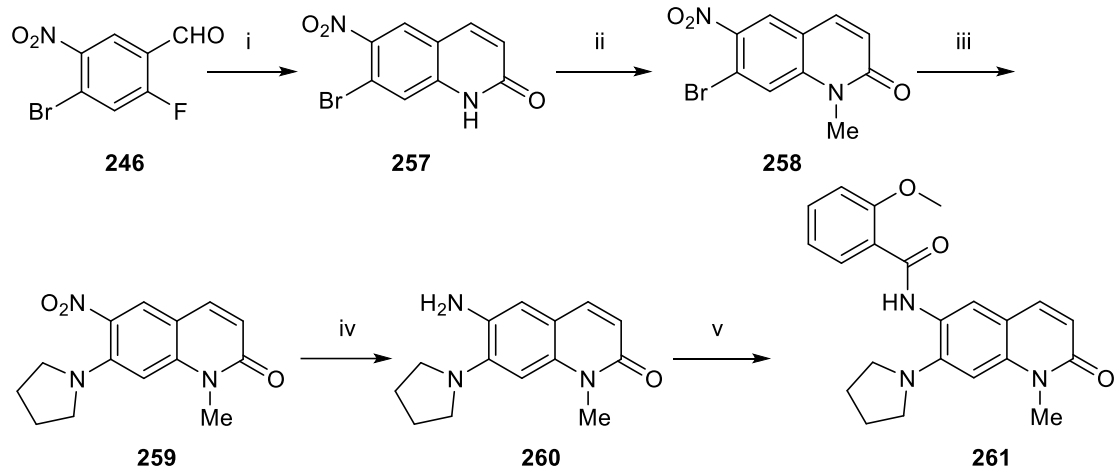
Scheme 41. Reagents and Conditions: i) Cs_2CO_3, DMF, 1,4-diiodobutane, 57%; ii) NBS, DMF, 12%.

The initial strategy focussed on reduction of the nitro group from compound **249** to give aniline **253** which could then be converted into the pyrrolidine by heating with base and 1,4-diiodobutane. Disappointingly the reduction of the nitro group either failed to yield the amine, or gave an intractable mixture of products, depending in conditions employed.

Therefore, the alternative strategy of cyclising the amine **129a** prior to bromination with NBS was investigated. Cyclisation was required prior to bromination as previous work in our lab had shown

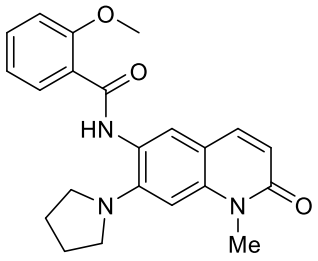
that bromination of **129a** exclusively provided the undesired 5-Br regioisomer. Cyclisation with 1,4-diiodobutane with Cs_2CO_3 yielded the pyrrolidine in good yield, however reaction of **254** with NBS was extremely slow, failing to go to completion regardless of the number of equivalents of NBS and reaction time. Therefore **255** was isolated in only 12% yield. Attempts at cross coupling with 2-methoxybenzamide (**256**) were unsuccessful, as were attempts at installation of an amine group *via* the previously employed Cu catalysed microwave amination

It was hoped that one method for promoting the correct conformation of the quinolinone would be to remove the methyl group at the 3 position. A synthetically identical procedure to that used for **252** was utilised, which is outlined in scheme 42



Scheme 42. Reagents and Conditions: i) $NaHSO_4$, 2,4,4-trimethyl-4,5-dihydrooxazole, Xylene, NMP RT - 110 °C *then* 110 - 210 °C, 66%; ii) NaH, MeI, DMF, 17%; iii) pyrrolidine, CuI (cat), DMF, μw 100 °C, 77%; iv) $SnCl_2$, conc. HCl, 77%; v) RCOCl, pyridine, DMF, quant.

Substituting the 2-ethyl-dihydrooxazole for the methyl analogue afforded the unmethylated quinolinone which was subjected to the same conditions as for scheme 40 for pyrrolidine substitution, reduction and coupling



MW 377
tPSA 62 Å^2
ClogP 2.55

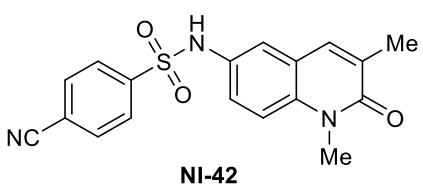
BRD	K_D (nM)	Selectivityof 261 *vs* BRPF1 (× fold)	Selectivityof 261 *vs* BRPF1 (× fold)
BRPF1	**760**	**-**	**-**
BRD1	**> 25,000**	**> 33**	**12**
BRPF3	**> 25,000**	**> 33**	**55**
BRD9	**2,900**	**4**	**1.9**
BRD4	**8,300**	**11**	**42**

Table 41. Biochemical and biophysical evaluation of compound **261**. a. T_m carried out by Dr Oleg Fedorov at the SGC. Experiments carried out at 10 μM, n = 1. b. K_D's determined by DiscoverX. Most K_D's were determined using a compound top concentration = 10,000 nM. Dose response curves were determined at an 11 point serial dilution. If the initial K_D determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. Data is the mean of 2 replicates

Amide **261** is less potent, but in general it is more selective against the class IV BRDs. However, the selectivity against the key selectivity target BRD4 is significantly lower. As a consequence of its low inhibition of BRPF1 this series of inhibitors was not investigated further.

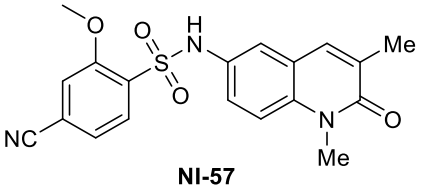
5. NI-57 and NI-42: BRPF Probes

With their favourable potency and selectivity profiles **NI-42** and **NI-57** were both deemed suitable for use as BRPF biased and BRPF specific probes respectively of the class IV BRDs (Tables 21 and 26 respectively). Initially **NI-57** was confirmed to engage with BRPF1 in an intracellular setting before both compounds their pharmacokinetic (PK) profiles extensively profiled. Subsequently, both compounds were employed in *in vitro* and *in vivo* settings to determine the consequences of BRPF BRD inhibition.



NI-42

MW 353
tPSA 90
ClogP 2.46



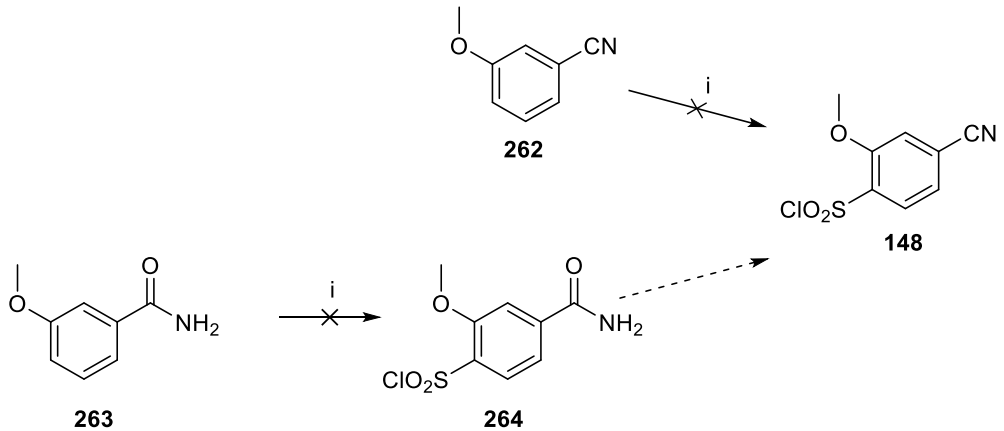
NI-57

MW 383
tPSA 99
ClogP 2.08

5.1 Large Scale Synthesis of NI-57

Whilst synthesis of the 6-amino-1,3-dimethylquinolin-2(1*H*)-one (**129a**) core for **NI-57** (Scheme 44) was reasonably efficient (21% over 5 steps, chromatography free), the capricious nature of the sulfonyl chloride synthesis and difficulty in product purification required improvement. This would allow the synthesis of **NI-57** on the scale necessary to be able to provide the material for biological evaluation.

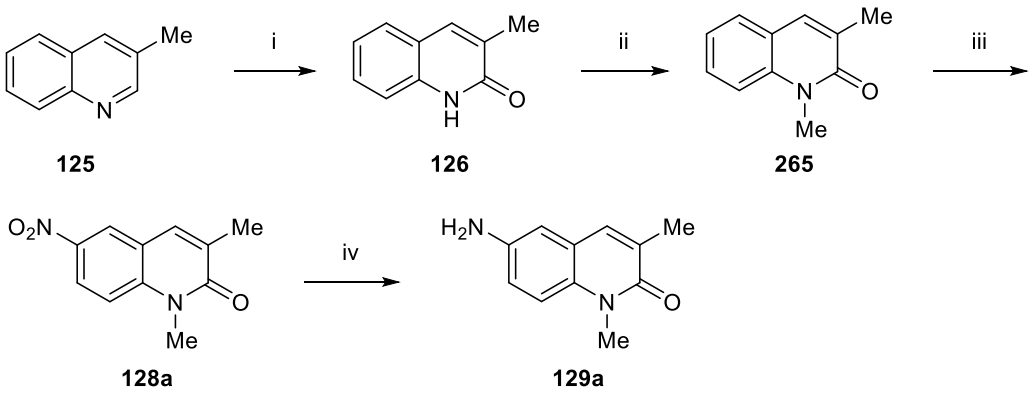
Thus, alternate strategies were investigated for the synthesis of 2-methoxy-4-cyanobenzenesulfonyl chloride (**148**). It was hoped that with a cleaner product profile for the sulfonyl chloride it might be possible to carry out purification of the target sulfonamide without the need to employ column chromatography.



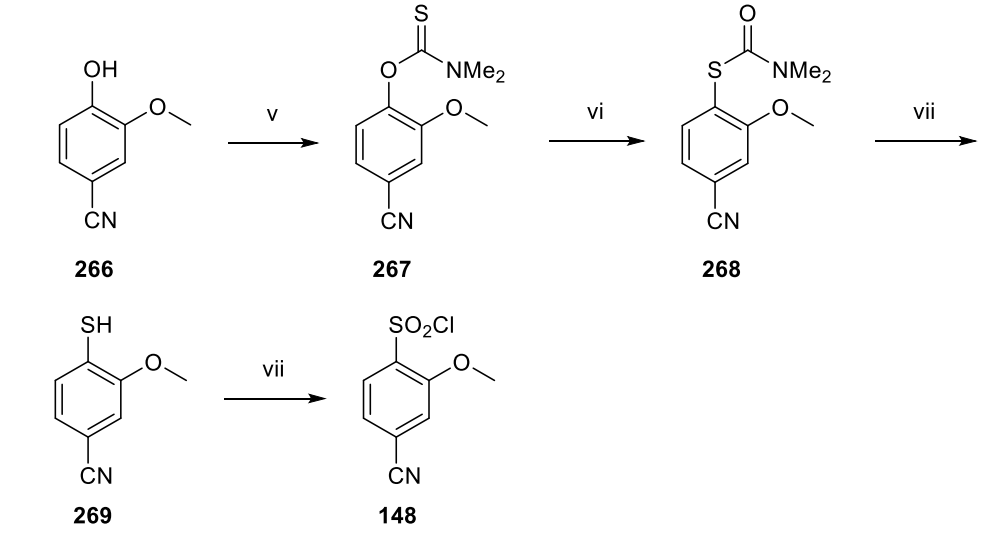
Scheme 43. Reagents and Conditions: i) HSO_3Cl, 40-60 °C

Initially 3-methoxybenzonitrile (**262**) was treated with HSO_3Cl at 0 °C, however no reaction was observed so the reaction mixture was then heated to 40 and then 60 °C, leading to a complex mixture of products. Literature synthesis of the structurally similar 3-methoxy-4-cyanobenzenesulfonyl chloride involved reaction of the primary formamide with HSO_3Cl, followed by dehydration of the amide to provide the nitrile. Adaptation of these conditions also failed to provide any of the desired sulfonyl chloride. [102]

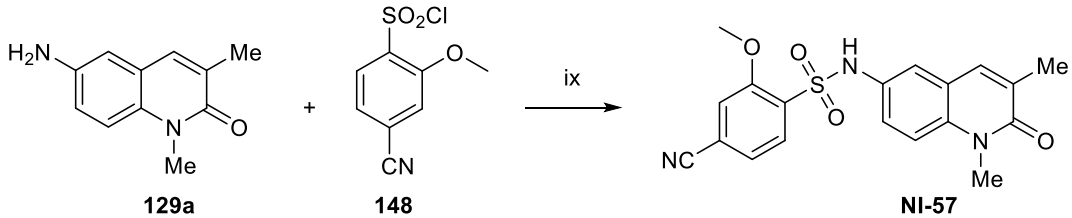
OPTIMISED SYNTHESIS OF 6-AMINO-1,3-DIMETHYLQUINOLIN-2(1*H*)-ONE CORE



OPTIMISED SYNTHESIS OF 2-METHOXY-4-CYANOBENZENESULFONYL CHLORIDE



OPTIMISED COUPLING FOR SYNTHESIS OF NI-57



Scheme 44. Reagents and Conditions: i) *m*CPBA, CH_2Cl_2 *then* Benzoyl chloride, NaOH (1.0 M), CH_2Cl_2, 59% over 2 steps; ii) NaH, MeI, DMF, 95%; iii) H_2SO_4, HNO_3, 0–5 °C, 87%; iv) H_2, Pd/C, EtOH, 95%; v) dimethylthiocarbamoyl chloride, DABCO, DMF, 50 °C, 76%; vi) 200 °C, 94%; vii) KOH, MeOH, 30 °C, 95%; viii) $ZrCl_4$, H_2O_2, MeCN, 0 °C, 92%; ix) DABCO, DMF, 76%.

Eventually, work carried out in our lab (Scheme 44, work carried out by Dr. Elliott Bayle, checking in progress for *Org Synth*) devised a 4 step synthesis of **148** starting from 2-methoxy-4-cyanophenol (**266**) involving a key Newman-Kwart rearrangement, followed by hydrolysis and oxidation to provide the required sulfonyl chloride. Moreover modification of the coupling conditions, by reducing the equivalents of sulfonyl chloride and replacing pyridine with DABCO, afforded analytically pure **NI-57** without the need for purification.

5.2 On-target engagement

Having confirmed the potency and selectivity of **NI-57** in biophysical assays we next sought to confirm that the compound engaged with the BRPF bromodomains in an *in vitro* setting.

The first assay run was a cellular thermal shift assay (CETSA), which involved incubating cells with the inhibitor of interest before heating the cell to denature the protein, causing it to precipitate. The quantity of protein remaining in solution can then be determined by lysing the cells, centrifugation to remove denatured protein and subsequent analysis to quantify the amount of soluble protein. Similarly to TS, the inhibitor binding to the protein increases the protein stability. As a consequence the protein does not denature on heating and can thus be detected in greater quantities in the soluble protein fraction.

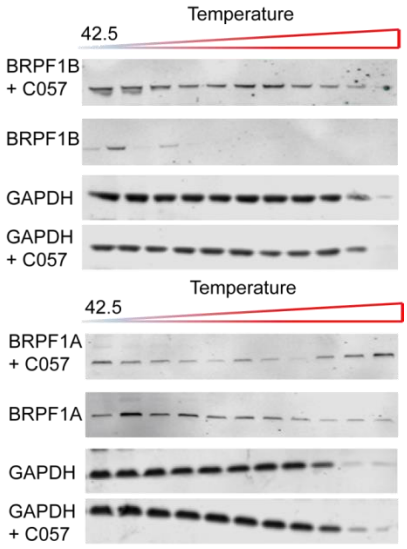


Figure 53. CETSA analysis of NI-57 showing binding to BRPF1B but not BRPF1A. GAPDH is used as a positive control. Work carried out at the SGC (C057 is the compound ID for **NI-57** in this experiment

Analysis of the western blot indicates that NI-57 interacts with BRPF1B, but not the inactive splice variant BRPF1A. This can be seen by the disappearance of BRPF1A from the soluble fraction with the increase in temperature, which is not observed when **NI-57** is added to the cells (Figure 53).

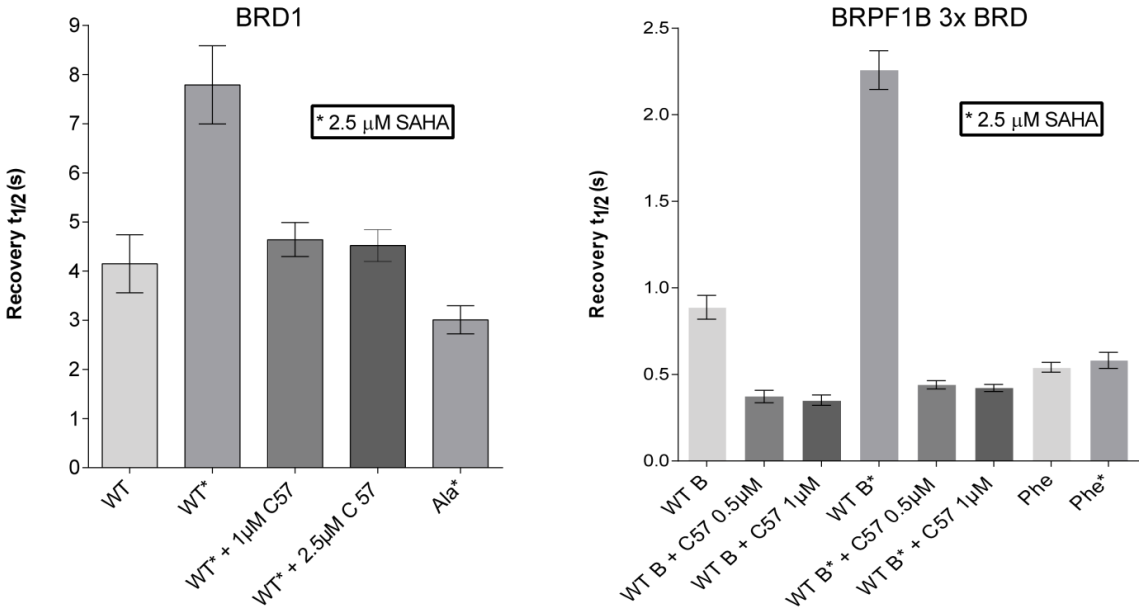


Figure 54. FRAP assay data confirming *in vitro* target engagement of NI-57 to BRPF1 and BRD1 at 1 μM. Work carried out at the SGC

Fluorescence recovery after photobleaching (FRAP) was utilised to confirm *in vitro* binding to BRD1 and further confirm BRPF1 binding. This assay assesses protein diffusion by monitoring the fluorescence of green fluorescent protein. After photobleaching the BRPF will diffuse back into the bleached area. The rate of diffusion is retarded by BRD binding to acetylated chromatin. If however, the BRD is bound to an inhibitor the recovery time is significantly reduced. Introduction of SAHA (an HDAC inhibitor) increases the acetylated lysine levels, increasing recovery time and exacerbating the difference between the cells with and without the inhibitor. For both bromodomains there was accelerated recovery after photobleaching (BRD1: ~2 fold faster recovery at 1 μM, BRPF1: ~2 fold faster recovery at 1 μM) indicating that **NI-57** is inhibiting the association of BRPF1/BRD1 to the chromatin in the nucleus (Figure 54).

5.3 Pharmacokinetic Profiles

Prior to investigation of *in vivo* effects of BRPF inhibition it was necessary to confirm that both **NI-42** and **NI-57** possessed suitable PK profiles. The first parameter tested was cellular permeability *via* a Caco-2 assay (Table 41).

Parameter	NI-42	NI-57
A to B (10^{-5}cm^{-1})	9.94 (2.53)	11.3 (0.80)
B to A (10^{-5}cm^{-1})	49.6 (0.60)	52.0 (8.87)
Efflux Ratio	4.99	4.61

Table 42. Caco-2 assay results for **NI-57** and **NI-42**. Assay carried out at 10 μM at 37 °C, data is presented as the mean of two results with the standard deviation in brackets. A to B is apical to basolateral layer and B to A is basolateral to apical layer.

The results show that both compounds are cell permeable, showing excellent apical to basolateral (A to B) diffusion, suggesting that ~90% of both compounds would be absorbed in the intestine. [65] However, given that the efflux ratio (B to A/A to B) is greater than 2, it is possible that the compounds are substrates for active efflux from the cell. Further investigation, involving remeasurement of the data in the presence of an active efflux inhibitor, would be required to confirm the hypothesis. [65]

Having confirmed that the compounds were cell permeable we next determined *in vitro* compound stability, by measuring both human and murine liver microsomal stability. Data from the assay would help determine the phase I stability of both compounds by assessing the extent to which they are metabolised by the cytochrome P450 (CYP) proteins.

Animal	NI-42 Cl_{int} (ml/min/g of liver)	NI-57 Cl_{int} (ml/min/g of liver)
Mouse	0.8	0.2
Human	0.3	1.2

Table 43. Human and Mouse liver microme stability studies for **NI-42** and **NI-57**. Work carried out by Anne Cheasty at Cancer Research UK

Both compounds showed low clearance in both mice and human liver microsomes. In particular the value for human microsomal stability is promising, with values below 8.6 considered low clearance. [103] This bodes well for their *in vivo* stability (Table 42).

In order to be able to traverse the cell membrane it is important that the compound is not bound to the plasma proteins in the blood.

Animal	NI-42 PPB (%)	NI-57 PPB (%)
Mouse	99.0 (0.2)	98.9 (0.4)[a]
Human	96.8 (0.4)	100 (0.0)[a]

Table 44. Human and Mouse plasma protein binding studies studies for **NI-42** and **NI-57**. Work carried out by Anne Cheasty at Cancer Research UK. a. Due to 50 ng/mL limits of detection by LCMS the values for NI-57 may vary from true values.

Analysis of the plasma protein binding (PPB) in humans and mice suggests that both compounds have high PPB percentages, although **NI-42**'s values are not as high as those of **NI-57.** Furthermore, the levels of PPB for **NI-57** may not be as high as recorded, as sensitivity issues were encountered with the LCMS analysis for this assay. Indeed the US Federal Drug Administration recommend that when PPB free drug fractions of less than 1% are recorded that the value be arbitrarily set at 1% as a consequence of uncertainty at these low values. [104]

Having confirmed that both compounds had promising initial *in vitro* PK profiles *in vivo* data was collected to support future experiments. Both compounds were tested by intravenous injection (IV 1 mg/kg 5 mL/kg) and orally (P.O. 3 mg/kg).

IV 1 mg/kg, 5 mL/ kg

Parameter	NI-42	NI-57
Cmax (μM)	6.5	2.3
Clb (mL/min/kg)	4	21
Vss (L/kg)	0.7	1.3
t1/2 (h)	2.0	1.2
MRT	2.6	1.0

PO 3 mg/kg

Parameter	NI-42	NI-57
Cmax (μM)	3.95	0.63
Tmax (h)	1.0	2
t1/2 (h)	1.7	1.6
$AUC_{0\text{-}7h}$ (ng.h/ml)	5692	651
$\%Fpo_{(0\text{-}\infty)}$	49	29

Table 45. *In vivo* mouse PK for **NI-42** and **NI-57**. Studies carried out on Female CD-1 mice, data the average of 2 replicates. Work carried out by Anne Cheasty at Cancer Research UK.

Following an intravenous dose of 1 mg/kg **NI-57** shows low clearance (21 ml/min/kg) with a reasonable 1.4 l/kg volume of distribution. The low *in vitro* clearance value of 0.2 ml/min/kg combined with the t½ of 1.2 hours suggest that the compound possesses good mouse *in vitro* stability.

Oral dosing of 3 mg/kg highilighted that **NI-57** had a maximum concentration (C_{max}) of 0.63 μM after 2 hours (T_{max}). The area under the curve between 0 and 7 hours ($AUC_{0\text{-}7h}$) was calculated as 651 ng.h/ml, whilst the oral bioavailability was 29%.

For **NI-42** the IV clearance was even lower than **NI-57,** at 4 mL/min/kg, although the Vss was slightly lower at 0.7 L/kg. As a consequence of the low clearance the t½ was slightly longer at 2h, but similarly to **NI-57, NI-42** posesses very low *in vivo* clearance.

Following oral administration of **NI-42** a Cmax of 4 μM was observed at 1 hour. This allowed for the calculation of an oral bioavailability of 49% and a $AUC_{0\text{-}24h}$ of 5692 ng.h/ml. Taken as a whole, the oral and IV administration profile of **NI-42** was superior to that of **NI-57.**

The CYPs play a major role in drug metabolism. As such, it is important to assay which CYPs a drug interacts with, as compounds with similar CYP profiles can effect one anothers plasma levels over time. Thus, to avoid unfavourable drug-drug interactions it is vital to know which CYPs a compound inhibits.

CYP	NI-42 IC_{50} (μM)	NI-57 IC_{50} (μM)
CYP2C9	0.854 (0.155)	4.80 (0.406)
CYP1A	> 25	> 25
CYP3A4	> 25	3.35 (0.685)
CYP2D6	22.1 (5.13)	> 25
CYP2C19	3.67 (0.622)	> 25

Table 46. Cytochrome P450 inhibition for **NI-42** and **NI-57**. Data is presented as the average of 2 replicates with standard deviation in brackets. Work carried out at Cyprotex.

In comparison to their ITC K_D's the CYP inhibition of both compounds was minimal, with most inhibitions being greater than 1,000 times the K_D of the inhibitor for BRPF1. The exception was **NI-42** and CYP2C9 for which **NI-42** had an IC_{50} of 854 nM (Table 45). The above CYPs were chosen as they represent 5 of the 7 major human CYP isoforms. [65]

5.4 Safety

Having assessed the probe PK profiles, cardiac ion channel inhibition was next investigated to determine compound safety. The most important of the channels assayed was hERG, inhibition of which can lead to fatal tachyarrhythmia. A number of other major ion channels were also tested (Table 46).

Ion Channel	NI-42 % Inhibition (30 μM)	NI-57 % Inhibition (30 μM)
Nav1.5	3.1	6.2
Kv4.3/KChIP2	25.9	14.9
Kv1.5	10.9	11.9
KCNQ1/minK	18.7	6.7
hERG	45.6	37
Cav1.2	9.0	6.4
Kir2.1	3.2	- 2.9
HCN4	- 0.7	- 2.2

Table 47. Ion channel inhibition of **NI-42** and **NI-57** at 30 μM. Data is presented as the average of 12 replicates. Work carried out at Eurofins

Determination of inhibition at a single concentration (30 μM) showed that the inhibition of ion channels in both compounds was pleasingly low.

NI-57 off-target selectivity was determined by a screen of 56 different kinases and GCPRs by CEREP. There was no significant binding at the test concentration of 10 μM, with the highest observed inhibition being 37% (see Apendix).

6. Biological evaluation

Having confirmed their suitable biochemical profiles (potency, selectivity, *in vitro* target engagement etc) and PK profiles **NI-42** and **NI-57** were investigated in a number of *in vitro* and *in vivo* experiments to determine the biological consequences of BRPF BRD inhibition.

6.1 BIOMAP

BIOMAP is a proprietary screening platform operated by DiscoverX which profiles a compound in more than 30 human derived disease cell lines and 48 disease models. The assay gives more than 200 readouts indicating areas of potential biological effect. The combined fingerprint of these biological effects can then be compared to the database of known drugs to suggest a potential mode of action.

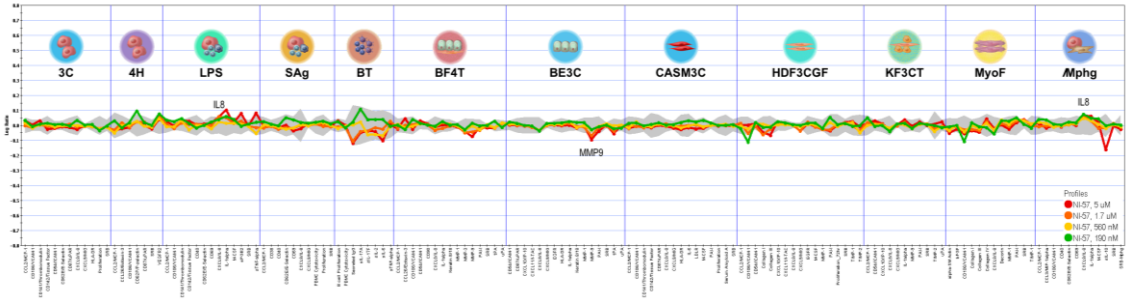


Figure 55. BIOMAP profile of **NI-57**

When profiled in this comprehensive assay **NI-57** failed to give any substantial readouts. There was a modest increase in IL-8, which is involved in inflammation, and MMP9, a protein involved in tissue remodelling, was also slightly activated. The data on the whole suggests that the global effects of BRPF1 bromodomain inhibition might be minimal. However, the assay also indicates that **NI-57** has a safe *in vitro* profile.

6.2 COPD

In collaboration with AstraZeneca **NI-57** was utilised to investigate the role BRPF inhibition might play in chronic obstructive pulmonary disease (COPD). COPD is characterised by progressive and irreversible airflow limitation and one of the potential causes is thought to be epigenetic changes.

It was hoped that inhibition of the BPRF bromodomains might reduce the immune response and subsequent inflammation caused by M-2 macrophages. *In vitro* analysis of **NI-57** showed that there was a decrease in M2 associated genes CCL-17 and CCL-22, suggesting that BRPF1 inhibition could reduce the inflammation caused by COPD. [105]

6.3 Cancer Panels

BRPF has been implicated in a number of cancers, particularly AML. **NI-57** was screened in both the National Institute of Health (NIH) cancer panel and a proprietary AstraZeneca cancer panel.

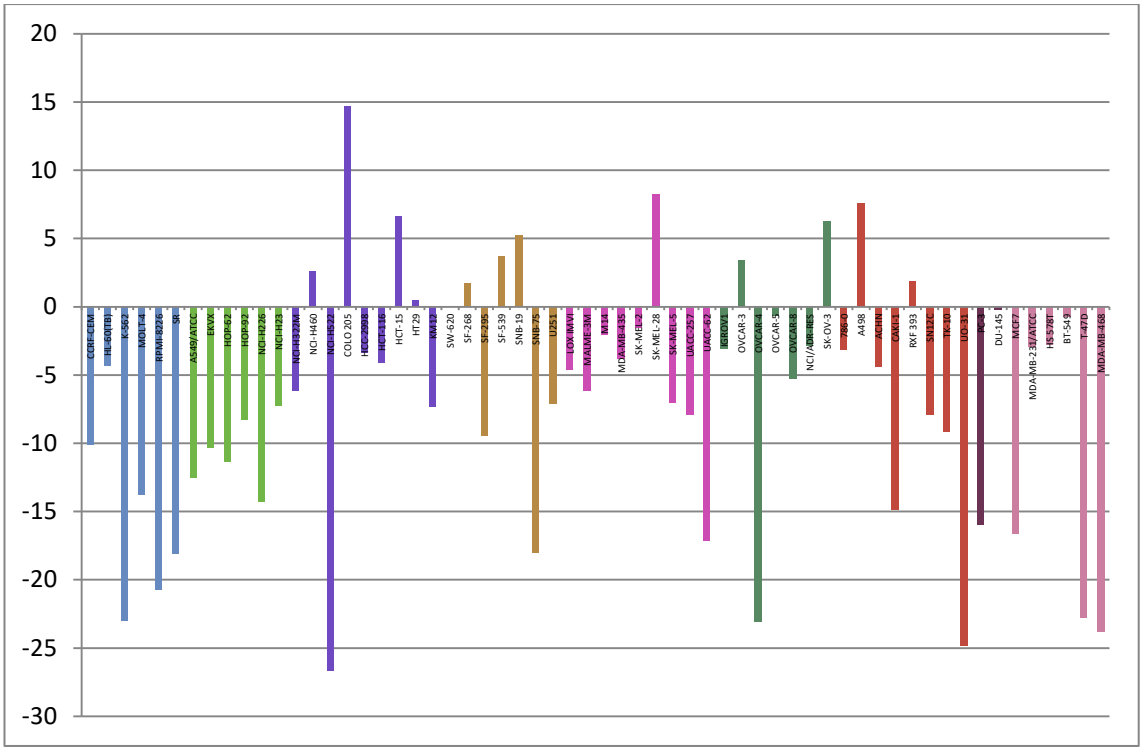


Figure 56. NCI-60 cancer cell line panel showing inhibition of cancer cell line growth at 10 μM. Different colors are indicative of different types of cancer, leukemia, lung cancer, colon cancer, CNS cancer, melanoma, ovarian, renal, prostate, breast

Growth inhibition for the NCI cancer panel was run at a concentration of 10 μM. As such, even the highest inhibitions recorded, around 25%, were modest. Although the data displayed no clear trends, there was a suggestion that AML cell line growth may be preferentially arrested by BRPF inhibition.

These findings are in line with the associations between BRPFs and this types of cancer and prompted screening in a more targeted AML cell line panel at Cancer Research UK to confirm these findings (Table 47).

Compound	THP1	MV 4:11
NI-42	50 μM	10-20 μM
NI-57	25 μM	25 μM

Table 48. GI_{50}'s from CRUK AML cell lines

Whilst inhibitions were modest, it was hoped that a larger screen of cell lines would prove more revealing. Thus both **NI-42** and **NI-57** were screened in AstraZeneca's more comprehensive cancer cell line panel (CLIMB panel). The results from this panel were disappointing for **NI-57** indicating no GI_{50} values below 10 μM (Figure 57). **NI-42** performed slightly better, recording values below

5μM in 12 cell lines. Further analysis of the data, focussing on the AML cell lines, highlighted that **NI-42** modestly and selectively inhibited AML cancer cell lines.

AML Cell Line	NI-42 GI_{50} (μM)
OCI - AML2	1.3
Nomo-1[a]	4.6
THP-1[a]	5.7
KG-1	7.0
MV-4-11[a]	9.9

Table 49. NI-42 inhibition of AML cell lines a. cancer cell lines exhibit MLL translocations

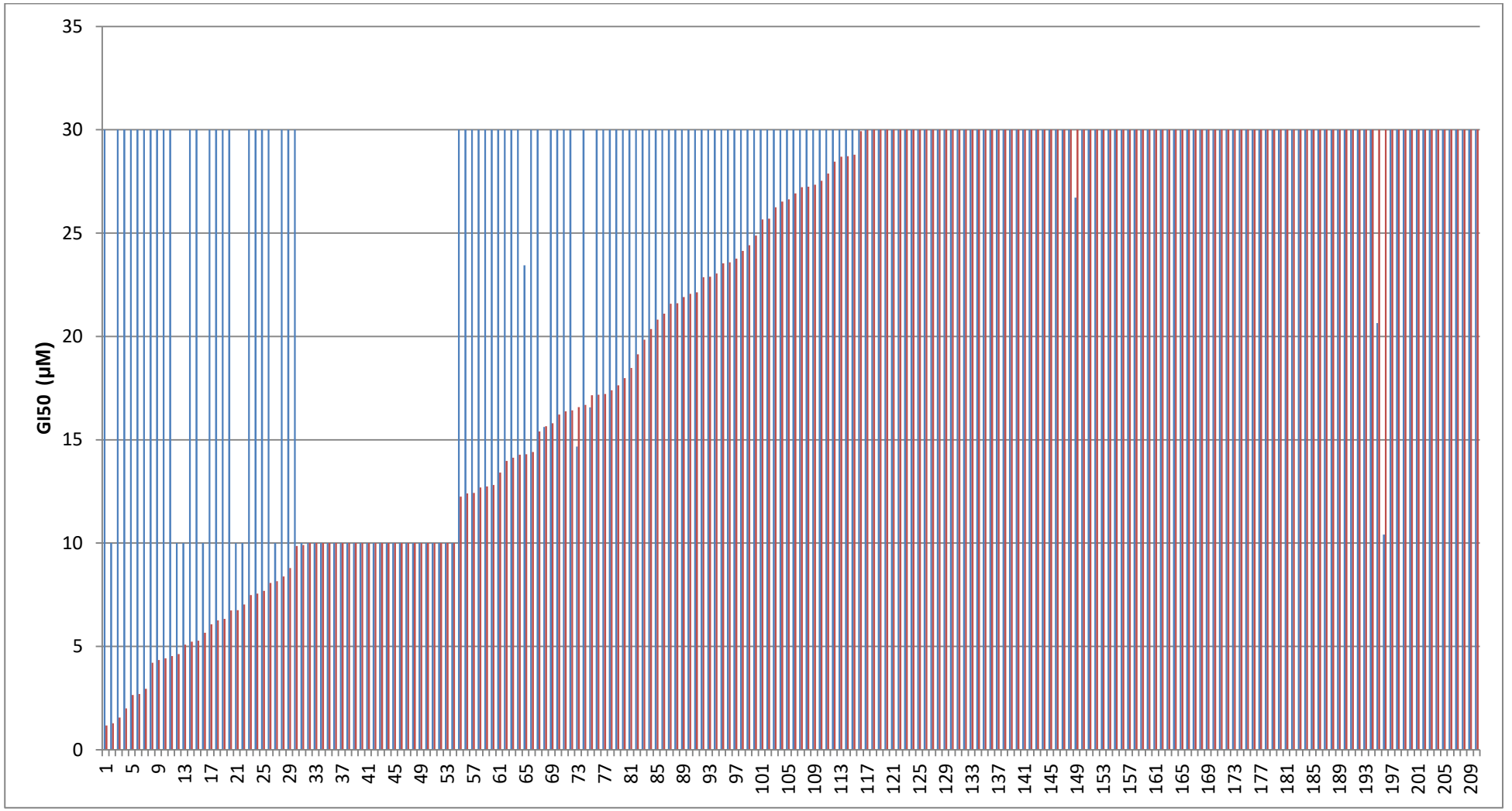


Figure 57. AZ CLIMB panel for **NI 42** (Red) and **NI-57** (blue. Cancer cell lines ranked by the inhibition of **NI-42,** with the strongest inhibition on the left

6.4 BRPF Inhibition and Osteoclastogenesis

NI-57 and its potential role in preventing osteoclastogenesis was investigated by the SGC by incubating the probe with bone marrow mononuclear cells which were then induced to differentiate. Inhibition of osteoclastogenesis could provide treatments for diseases caused by osteoclast overexpression, such as osteoporosis.

Incubation of **NI-57** with bone marrow cells caused significant reductions in the number of multinucleated TRAP-positive cells. Further analysis of biomarkers that signify inhibition of osteoclastogenesis found significant reductions in levels of: tartrate-resistant acid phosphatase (TRAP), the late-phase osteoclast marker cathepsin K (CATK), the proton generator carboanhydrase II (CA2), the key transcription factor NFATC1 and OC-STAMP, an essential cell-cell fusigen. Overall, the data indicated that inhibition of the BRPF BRDs caused significant reductions in osteoclastogenesis.

Significant reductions of MMP9 secretion were also noted, an observation consistent with the BIOMAP results. MMP9 is a protein that is secreted by osteoclasts to promote bone matrix degradation, which is of potential interest as MMP9 levels are upregulated in a number of invasive tumors.

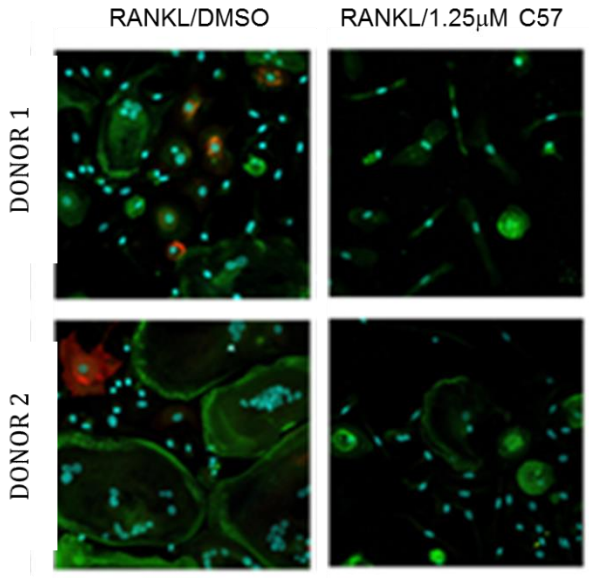


Figure 58. Imaging showing **NI-57** inhibition of osteoclastogenesis. Blue cells indicate TRAP-positive cells On the left DMSO control on the right dosing with **NI-57**. Work carried out by Julia Maier at the SGC

7. Conclusion

When we initiated our research project there was a clear link between the BRPF proteins and disease. However, there were no small molecules available to the scientific community to determine the role that the BRPF BRDs play in this disease association. As such, we set out, in collaboration with the SGC, to design and synthesise a fit for purpose, selective potent and cell permeable probe for the BRPF BRDs to validate this link.

Starting from the *N*-methylquinolin-2(1*H*)-one (**1**) core we have developed a consistent SAR model, by investigation of all the possible positions on both of the quinolone rings and the sulfonamide aryl ring, both in isolation and combination (Figure 59). Our work culminated in the synthesis of **NI-42** and **NI-57,** BRPF biased and BRPF specific BRD probes respectively. Following on from this we were able to modulate the activity and design inactive analogue **NI-198.**

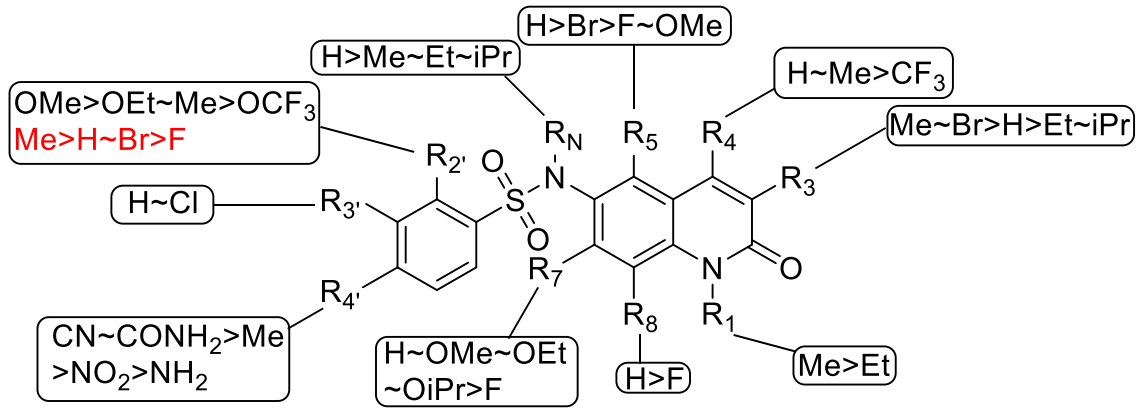


Figure 59. SAR Model generated for *N*-methylquinolin-2(1*H*)-one (**1**) core modifications on the potency against and selectivity for BRPF1. Substituent effect is primarily measured by potency. Comparisons made where possible by comparing identical cores. Different colours indicate comparison was made in reference to a different core

With **NI-57** having satisfied our initial goals of meeting the SGC criteria (30 fold selectivity against members of the same family, > 100 fold selectivity otherewise, potency < 100 nM and *in vitro* target engagement at < 1 μM), we next fulfilled the more stringent criteria outlined by Workman *et al* (Figure 61). [50b]

7.1 Further work

Despite strong evidence that aberrant behaviour of the BRPF proteins is involved in a number of aggressive leukaemias, incubation of both **NI-42** and **NI-57** produced only modest effects in several AML cell lines. As such, it is possible that BRPF protein's primary role is as a scaffolding protein for its endogenous tetramer.

During the course of our studies Winter *et al* proposed tethering BET inhibitor JQ1 (see above) to a phthalimide linker, which on binding to the BRD will target the protein for E3-ligase promoted degradation (Figure 60) . [106]

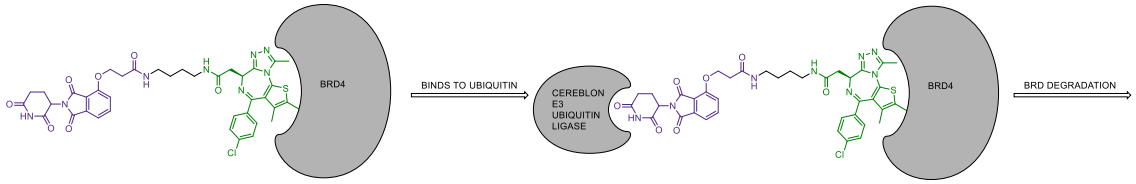
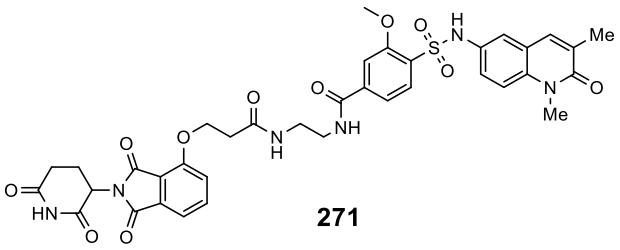


Figure 60. Strategy of Winter *et al* for promoting BRD degradation. JQ1 in green, pthalimide for E3 ligase binding in purple

By tethering **NI-57** to a similar phthalmide conjugate it will be possible to investigate whether cellular degradation of BRPF will have the desired effect on AML cell lines. This could easily be achieved by conjugation of the tether to the primary formamide of **NI-57** to give compound **272**, 4-(N-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)sulfamoyl)-3-methoxybenzamide **– 270,** BRPF1 K_D: 5 nM, which can be synthesised in high yield without purification from **NI-57.**

Furthermore by investigating the effects of BRPF protein degradation with pan BRPF and BRPF1 specific probes it may be possible to elucidate whether the BRPF proteins have redundant functions.



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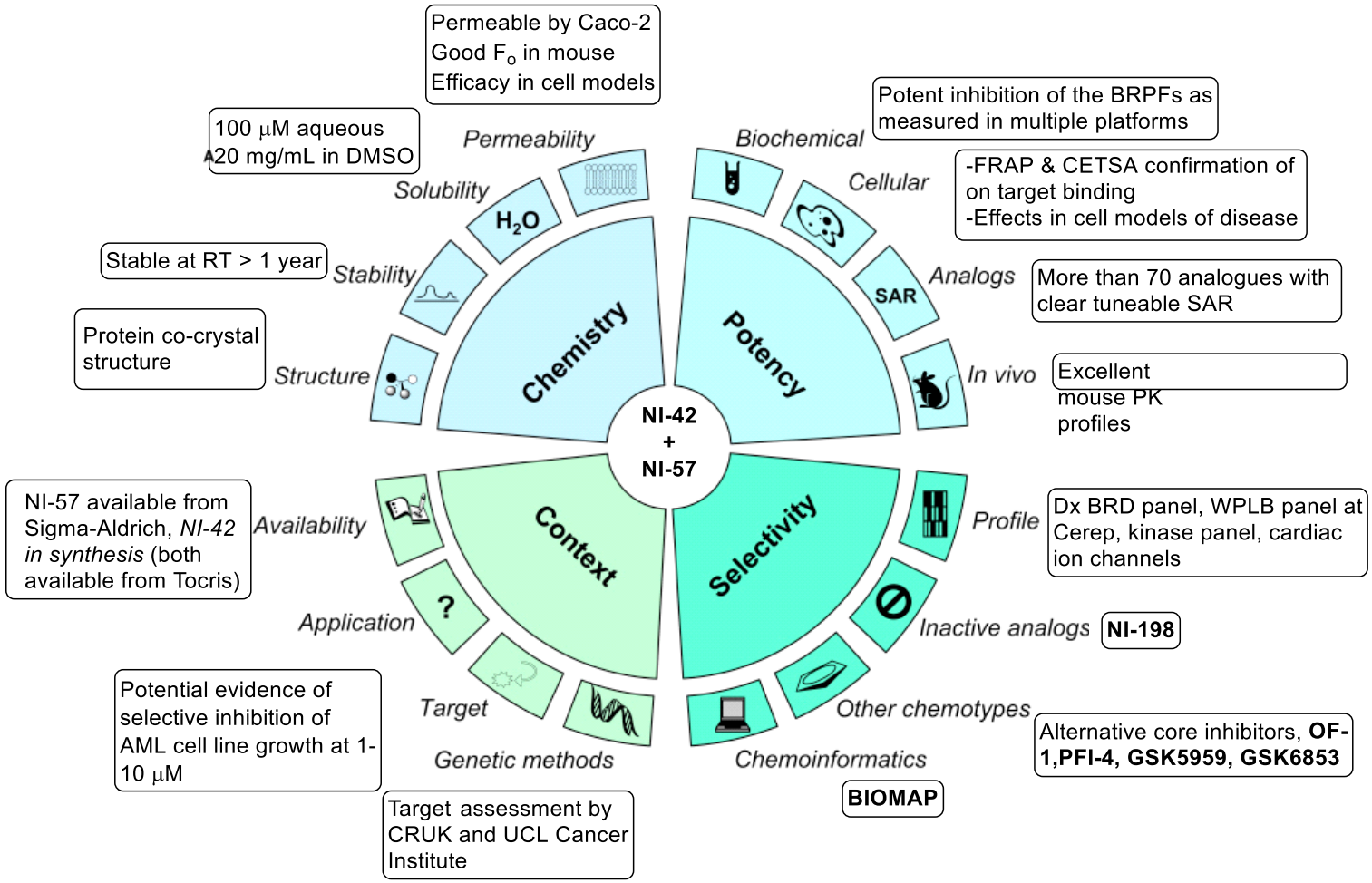
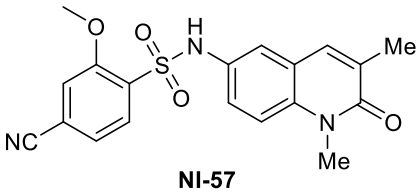


Figure 61. Probe criteria of Workman *et al* as demonstrated by **NI-57** and **NI-42.** [50b]

NI-57 PROBE PROFILE



NI-57

MW 383
tPSA 99
ClogP 2.08

Scalable, high yielding chromatography free synthesis

TARGET ENGAGEMENT

- X-Ray crystal structure confirmation of compound binding
- CETSA assay confirms *in vitro* BRPF1 binding
- FRAP confirms BRD1 and BRPF1 binding

PK

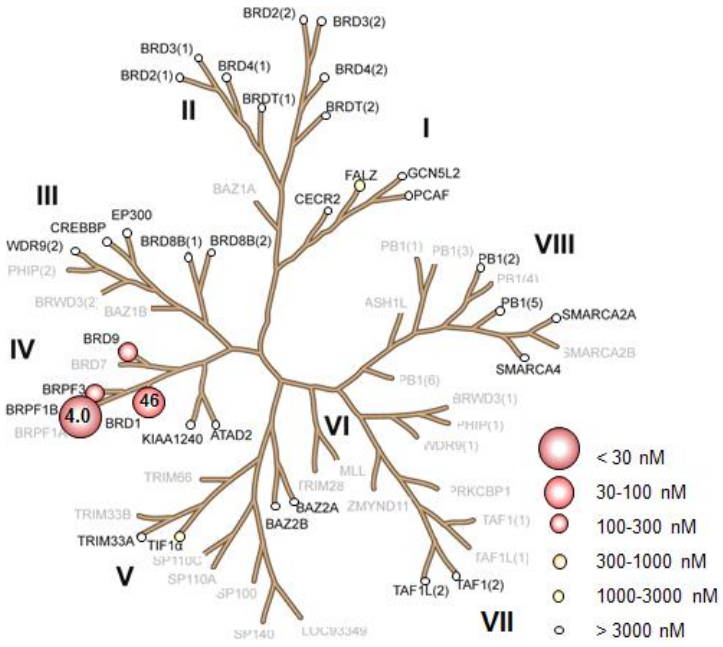
- Cell Permeable by CACO2 assay
- Modest CYP inhibition
- No ion channel inhibition
- No inhibition of GCPRs and kinases
- High PPB binding
- Good IV and PO profiles
- High metabolic stability, *in vivo* and *in vitro*.

Clb - 21 mL/min/kg ($t_{1/2}$ 1.2h)

POTENCY AND SELECTIVITY

BRD	ΔT_m (°C)	Dx K_D (nM)	ITC K_D (nM)	ITC K_D Selectivity *vs* BRPF1
BRPF1	10.7	4	31	-
BRD1	5.6	46	108	3.5
BRPF3	5.3	110	408	13
BRD9	3.1	160	997	32

BROMOSCAN DATA



COMPARISON TO OTHER PROBES

- Alternative KAc mimetic - orthogonal confirmation of binding mode
- Different selectivity profile c.f. OF-1 has weak BRD4 binding
  GSK compounds and PFI-4 completely BRPF1 selective
- Improved biopharmacokinetic properties to probes with 1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one core

BIOLOGICAL INTERROGATION WITH NI-57

- Inhibition of osteoclastogenesis
- Possible effects at mitigating the consequences of COPD
- Minimal effect in NCI-60 or AZ proprietary cancer cell line screens

8. Experimental

All chemicals were purchased from commercially available sources and used without further purification unless otherwise specified. Organic solvents used such as ethyl acetate (EtOAc), dichloromethane (DCM), Tetrahydrofuran (THF), methanol (MeOH), ethanol (EtOH) were provided by Fisher Scientific, while *N*,*N*-dimethylformamide (DMF) 99.8% was purchased from Ratburn Chemicals. The deuterated $CDCl_3$ and DMSO-d_6 were provided by Sigma Aldrich. Dry solvents were purchased from Acros

^{1}H and ^{13}C NMR spectra were recorded at ambient temperature in deuterated solvents ($CDCl_3$ or DMSO-d_6) on a Bruker Avance 400 or 500 NMR Spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) reference using the residual protonated solvent as an internal standard (^{1}H: δ ($CDCl_3$) = 7.26 ppm, δ (DMSO-d_6) = 2.50 ppm and ^{13}C: δ ($CDCl_3$) = 77.0 ppm, δ (DMSO-d_6) = 39.4 ppm). Data for ^{1}H NMR is given as follows: chemical shift, integration, multiplicity, coupling constants (*J*, given in Hertz (Hz)). Splitting patterns that could not be interpreted or easily visualised were recorded as multiplets (m) or broad peaks (br). 2D NMR techniques HSQC, HMQC and HMBC) were also utilised for the assignment of ^{1}H and ^{13}C NMR signals.

The progress of reactions was monitored by thin layer chromatography (TLC) performed on Keiselgel 60 F_{254} (Merck) silica plates and visualised by exposure to UV light at 254 nm.

Infrared (IR) spectra were recorded on a PerkinElmer FT–IR Spectrum™ 100, while melting points were determined in open capillary tubes on a digital melting point apparatus SMP10 (Stuart™). IR absorption peak values are expressed in cm^{-1} and melting points are given in ˚C and are uncorrected.

High-resolution mass spectra (HRMS) were recorded on a Thermo Navigator mass spectrometer coupled to an HPLC instrument using electrospray (ES) ionisation and time-of-flight (TOF) mass spectrometry.

System A: Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out on a XSELECT™ CSH™ C-18 column 2.5 µm 6 x 50 mm. HPLC experiments were performed with gradient conditions: initial fixed composition 5% B to 50% B over 20 min, then increased to 95% B over 2 min, held for 2 min at 95% B, then returned to 5% B in 1 min. Total duration of gradient run was 25 min. Eluents used were solvent A (H_2O with 0.02% TFA) and solvent B (MeCN with 0.02% TFA). Flow rate: 1.00 mL/min

General Procedure A: Preperation of quinolin-2(1*H*)-ones from quinolines

To a stirred solution of quinoline (1 eq.) in DCM (1 mL/mmol) at 0 °C was added 3-chloroperbenzoic acid (77 % w/w, 1 eq.) portionwise over 10 minutes. The resulting solution was

allowed to warm to room temperature and then stirred overnight. After completion of the reaction the solution was washed with sodium hydroxide (1.0 M, 3 × volume of DCM) and the aqueous phase extracted with DCM (3 mL/mmol). The organic layers were combined and dried over *anhydrous* $MgSO_4$, filtered and the solvent removed under reduced pressure to yield the appropriate quinolone-*N*-oxide which was used in the next step without further purification.

To a stirred solution of quinolone-*N*-oxide in DCM (2 mL/mmol) was added sodium hydroxide (1.0 M, 1.5 mL/mmol) and the resulting biphasic mixture was cooled to 0 °C. To this was added, under rapid agitation, benzoyl chloride (1.2 eq.) dropwise. The suspension was stirred for 2 hours and the resulting precipitate was collected by filtration, washed with water (50 mL) and dried under vacuum to give the desired quinolinone.

General Procedure B: *N* alkylation of quinolin-2(1*H*)-ones

To a solution of quinolin-2(1*H*)-one (1 eq.) in dry DMF (2 mL/ mmol) under an argon atmosphere was added NaH (60% w/w, 1.2 eq.) in one portion. Upon the completion of gas evolution, the appropriate iodoalkane (1.2 eq.) was added in 1 portion and the resulting solution was stirred overnight. Excess sodium hydride was quenched by the addition of water (3 × vol. of DMF). If precipitation was observed the precipitate was isolated by filtration, washed with water and dried to afford the desired *N*-alklyquinolinone.

Otherwise, the solution was extracted with ethyl acetate (3 × vol. of DMF), washed with water and then brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and then concentrated under reduced pressure. The crude solid was purified by column chromatography to afford the desired *N*-alklyquinolinone.

General Procedure C: Nitration of quinolin-2(1*H*)-ones

To a suspension of the appropriate quinolinone (1 eq.) in concentrated H_2SO_4 (2 mL/ mmol) at –5 °C was added HNO_3 (70% w/w, 0.5 mL/mmol) dropwise. The resulting yellow solution was stirred at this temperature for 2.5 hours before being allowed to warm to room temperature. The solution was next poured over crushed ice and the resultant suspension stirred for 5 minutes. The precipitate was collected by filtration and dried under vacuum to give the appropriate 6-nitroquinolinone.

General Procedure D: Reduction of 6-nitroquinolin-2(1*H*)-ones with $SnCl_2$

To a suspension of the appropriate 6-nitroquinolinone (1 eq.) in concentrated HCl (5 mL/mmol) was added $SnCl_2{\cdot}2H_2O$ (5 eq.) and the resulting suspension was stirred overnight. Sodium hydroxide was added with cooling until the pH had reached ~pH 10. The aqueous solution was then extracted with DCM (3 × 100 mL) and the organic layers were combined and the solvent removed under reduced pressure to give the appropriate 6-aminoquinolinone.

General procedure E: Coupling of amines to sulfonyl chlorides

To a solution of amine (1 eq.) in DMF (0.2 M, minimum 1 mL) was added pyridine (2 eq.) and the resulting solution was stirred for 5 minutes and then sulfonyl chloride (1.5 eq.) was added. The resulting solution was stirred overnight and then diluted with acetone (~20 mL). Celite ® was added and the suspension was concentrated under reduced pressure. Purification of the crude solid by column chromatography afforded pure sulfonamide.

General procedure F: Coupling of amines to 1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride

To a solution of 1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride (100 mg, 0.39 mmol, 1.1 eq.) and pyridine (31 mg, 32 μL, 1.1 eq.) in DCM (5 mL) was added the appropriate amine (0.35 mmol, 1 eq.) and the resulting solution was stirred overnight. After this time the organic solvents were removed under reduced pressure and the residue purified by column chromatography (ethyl acetate: hexane) to give pure product

General Procedure G: Preperation of 6-nitroquinolin-2(1*H*)-ones from 2-halo-5-nitrobenzaldeyhdes

The appropriate 2-halo-4-nitrobenzaldehyde (1 eq.) and the appropriate 4,4-dimethyl-2-alkyl-4,5-dihydrooxazole (1 eq.) were dissolved in NMP (0.75 mL/mmol) and Xylenes (0.4 mL/mmol) and sodium bisulfate (0.1 eq.) was added. A Dean-Stark apparatus was attached and the mixture slowly heated to 200 °C and maintained at this temperature for 90 mins and then raised to 225 °C for 45 mins. After this time the reaction was allowed to cool to RT, added to water (10 × vol of NMP), filtered and dried to give the desired 3-alkyl-6-nitroquinolinone.

General Procedure H: Sulfonamide *N* alkylation

To a solution of sulfonamide (1.0 eq.) in dry DMF (3 mL) under an argon atmosphere, was added NaH (60% in mineral oil, 1.2 eq.) in one portion. Upon the completion of gas evolution, the appropriate iodoalkane (1.2 eq.) was added in 1 portion and the resulting solution was stirred overnight. Excess sodium hydride was quenched by the addition of water (30 mL) and the aqueous solution was extracted with EtOAc (3 × 50 mL). The organic fractions were pooled, dried over anhydrous $MgSO_4$, filtered and concentrated onto celite ®. Purification of the residue by column chromatography provided the desired *N*-alkylated sulfonamide.

General Procedure I: Oxidation of tetrahydroquinolin-2(1*H*)-ones with DDQ

To a solution of the appropriate of tetrahydroquinolinone (1 eq.) in dry DCE (5 mL/mmol) under an argon atmosphere was added freshly crystallised DDQ (3 eq.) and the resulting dark suspension was stirred at reflux for 3 hours before being allowed to cool to RT. NaOH (1.0 M, 25 eq.) was added with stirring and the mixture was extracted with EtOAc (3 × 100 mL). The organic fractions were pooled, washed with NaOH (3 × 100 mL), saturated brine, dried over anhydrous Mg SO_4, filtered and concentrated to provide the quinolinone which was either used without further purification or purified by column chromatography.

General Procedure J: Microwave conversion of bromides to amines

The appropriate arylbromide (1 eq.) was dissolved in NMP (1.5 mL) in a Biotage 10 mL microwave vial. Cu_2O (0.1 eq.) and NH_4OH (28–30% NH_3, 2 mL) were added and the vial was sealed and heated at 110 °C under microwave irradiation for 3 hours. After cooling to RT the solution was filtered through a pad of celite® and washed with DCM (20 mL). The filtrate was washed with an aqueous lithium chloride solution (0.5 M, 10 mL) and the organic fractions were combined and concentrated under reduced pressure. The resulting residue was purified by column chromatography to give a solution of the amine in NMP.

General Procedure K: Synthesis of Cinnamides:

A solution of the appropriate aniline (1 eq.) and potassium carbonate (1.5 eq.) in acetone (1 mL/mmol of amine) and water (2 mL/mmol of amine) was cooled to 0 °C and cinnamoyl chloride (1.25 eq.) was added portionwise over 10 minutes. Sitrring was continued for 1 hour at 0 °C and then warmed to RT and stirred for 1 hour. The product was isolated by filtration and either purified by crystallisation or used without further purification.

General Procedure L: Cyclisation of Cinnamides

A solution of the appropriate cinnamide (1 eq.) in chlorobenzene (7.5 mL/mmol) was cooled to 0 °C and aluminium chloride (3 eq.) was added portionwise. The resulting suspension was slowly heated to 120 °C and maintained at that temperature for 3 hours. After this time the solution was cooled to RT and poured over ice water. The precipitate was removed by filtration and dried to give the crude quinolin-2(1*H)*-ones which were either crystallised or used without further purification.

General Procedure M: Preparation of Quinolin-2(1*H*)-ones from 2-*N*-protectedbenzaldehydes

Step 1 To a solution of diisopropylamine (2.1 eq.) in dry ether (1.25 mL/mmol) under an argon atmosphere at –78 °C was added *n*-butyllithium solution (1.6 M in hexane, 2.1 eq.) and the solution was stirred for 30 minutes. *Tert*-butyl acetate (2.1 eq.) was added dropwise and the solution was allowed to stir for 30 minutes. The appropriate benzaldehyde (1.0 eq.) in dry ether (1 mL/mmol) was added dropwise and the bright yellow solution was allowed to warm to RT over 2 hours. Ammonium chloride solution (1.0 M, 20 mL) was added and the reaction mixture stirred for a further 10 minutes. The aqueous layer was separated and extracted twice with ether. The combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure to provide the β-hydroxyester, which was used without further purification in the next step.

Step 2 Crude β-hydroxyester was dissolved in 1,4 dioxane (1 mL/mmol of benzaldehyde from step 1) and HCl (3M, 1 mL/mmol of benzaldehyde from step 1). The solution was heated at reflux for 4 hours and then allowed to cool to RT. The precipitated product was collected by filtration and dried under vacuum to yield the quinolin-2(1*H*)-one.

General Procedure N: Reduction of Nitroaryl Compounds in MeOH

The appropriate nitroaryl (1 eq.) was dissolved in MeOH (5 mL/mmol) and $SnCl_2 \cdot 2H_2O$ (5 eq.) was added and resulting suspension heated at reflux for 4 hours. The solution was allowed to cool to RT, basified to pH 9 with sat $NaHCO_3$, filtered and extracted with EtOAc (3 × 50 mL) to give a crude solution of amine which was either purified or used crude in the next step.

General Procedure O: Synthesis of Sulfonyl Chlorides

Sulfonyl chlorides were prepared from the appropriate amines by adapting the procedure of Hogan *et al*.[78]

Step 1: Thionyl chloride (0.4 mL/mmol of amine) was added dropwise to rapidly stirred H_2O (2 mL/mmol) at -10 °C whilst maintaining the temperature below -5 °C (Caution addition of thionyl chloride is very exothermic). On completion of thionyl chloride addition the resulting solution was allowed to gradually warm to ~17 °C overnight and then freshly purified CuCl (0.1 eq.) was added to form a yellow green solution.

Step 2: The appropriate amine (1 eq.) was suspended in HCl (37% w/w, 1.5 mL/mmol) and heated to 50 °C for 45 minutes with rapid stirring. The resulting suspension was cooled to -5 °C and $NaNO_2$ (1.05 eq.) in the minimum amount of H_2O was added dropwise at such a rate that the internal temperature did not exceed 0 °C. The dark solution of aryldiazonium chloride was then stirred at 0 °C for 30 mins.

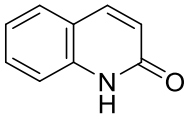
Step 3: The solution from step 1 was cooled to -5 °C (-15 °C for **146a** and **146b)** and then the solution from step 2 was added at such a rate that the temperature did not exceed 0 °C. On completion of addition the solution was stirred at 0 °C for 30 mins and then allowed to warm to RT and stirred until precipitation of the target sulfonyl chloride was observed. Sulfonyl chlorides were isolated by filtration, washed with water, dried under vacuum and then used without further purification in the next step.

General Procedure P: Synthesis of 2-(acrylate)-anilines

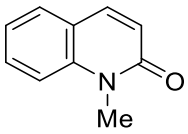
The appropriate 2-haloaniline (1 eq.), the appropriate acrylate (2 eq.) triethylamine (4 eq.), $Pd(OAc)_2$ (0.05 eq.) and $P(Ph)_3$ (2 eq.) were dissolved in dry DMF (5 mL/mmol) under an argon atmosphere and heated to 110 °C for 18 hours. After this time the solution was allowed to cool to RT, diluted with H_2O and extracted with EtOAc. The organic fractions were combined, washed with sat. brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the crude solid by column chromatography provided the pure acrylate.

General Procedure Q: Cyclisation of Acrylates

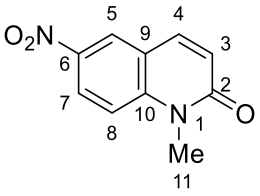
The appropriate acrylate was dissolved in dry MeOH (5 mL/mmol) and NaOMe (5 eq.) was added. The suspension was heated at reflux for 6 hours and allowed to cool to RT. The suspension was diluted with H_2O and filtered to provide analytically pure napthyridone.



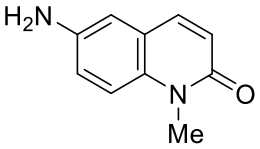
Quinlin-2(1*H*)-one (14): Prepared according to general procedure A from quinoline (1.0 g, 7.75 mmol) to give the title compound (0.88 g, 6.13 mmol, 78% over 2 steps) as a colourless solid: **mp** 195 – 196 °C (ethanol);**^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 11.75 (1H, br, s), 7.90 (1H, d, *J* = 9.5 Hz), 7.65 (1H, dd, *J* = 7.8, 1.6 Hz), 7.49 (1H, t, *J* = 8.1 Hz), 7.35–7.26 (1H, m), 7.22–7.09 (1H, m), 6.50 (1H, d, *J* = 9.5 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.7, 140.2, 138.8, 130.3, 127.8, 121.9, 121.7, 119.1, 115.1. The above data is in agreement with that previously reported in the literature. [107]



1-Methylquinolin-2(1*H*)-one (1) Prepared by general procedure B from **13** (530 mg, 3.63 mmol). Purification of the residue by column chromatography (3:2 ethyl acetate:hexane) gave the title compound (382 mg, 2.51 mmol, 69%) as a pale yellow solid: **mp** 70 – 71 °C (ethyl acetate-hexane) **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.91 (1H, d, *J* = 9.5 Hz), 7.72 (1H, dd, *J* = 7.7, 1.5 Hz), 7.67–7.61 (1H, m), 7.53 (1H, d, *J* = 8.5 Hz), 7.30–7.25 (1H, m), 6.62 (1H, d, *J* = 9.5 Hz), 3.62 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.3, 140.0, 139.0, 130.6, 128.8, 122.1, 121.8, 120.7, 114.1, 29.4. The above data is in agreement with that previously reported in the literature. [108]

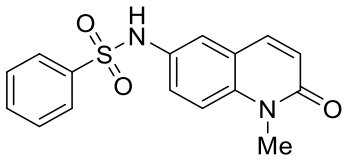


1-Methyl-6-nitroquinolin-2(1H)-one (15) : Prepared by general procedure C from **1** (1.20 g, 7.55 mmol) to give the title compound (1.34 g, 6.57 mmol, 87%) as a yellow solid: **mp** 218 - 219 °C (ethanol); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.75 (1H, d, *J* = 2.7 Hz, C_5H), 8.41 (1H, dd, *J* = 9.4, 2.7 Hz, C_7H), 8.16 (1H, d, *J* = 9.5 Hz, C_4H), 7.73 (1H, d, *J* = 9.4 Hz, C_8H), 6.81 (1H, d, *J* = 9.5 Hz, C_3H), 3.68 (3H, s, $C_{11}H$); **^{13}C NMR** (101 MHz, DMSO-d_6): δ ppm 161.7, 149.3, 140.9, 128.1, 124.6, 123.8, 123.3, 115.3, 62.1, 14.3. Data are in agreement with the literature. [67]

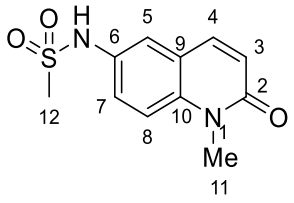


6-Amino-1-methylquinolin-2(1*H*)-one (2): Prepared according to general procedure D from **15** (180 mg, 0.88 mmol) to give the title compound (0.85 mmol, 97%) as a bright yellow solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.45 (1H, d, *J* = 9.5 Hz), 7.13 (1H, d, *J* = 8.9 Hz), 6.90 (1H, dd, *J* = 8.9, 2.7

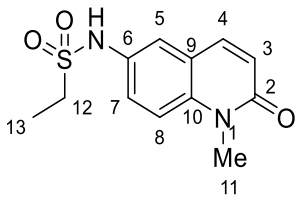
Hz), 6.76 (1H, d, *J* = 2.7 Hz), 6.60 (1H, d, *J* = 9.5 Hz), 3.66–3.58 (5H, m); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 160.9, 141.2, 138.1, 132.6, 122.3, 121.7, 119.4, 115.2, 112.8, 29.4.



***N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (3)** Prepared by general procedure E from **2** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane 3:7) to give the title compound (16 mg, 0.06 mmol, 47%): **mp** 201-204 °C (acetone-hexane); **IR** (neat) ν_{max}: 3080, 1641, 1581, 1485, 1310, 1157, 1091, 979, 989, 819, 754, 687, 592, 567, 506, 474 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 10.32 (1H, br. s), 7.83 (1H, d, *J* = 9.6 Hz), 7.76 (1H, s), 7.64–7.50 (3H, m), 7.46–7.39 (2H, m), 7.31 (1H, dd, *J* = 10.4, 2.0 Hz), 6.58 (1H, d, *J* = 9.4 Hz), 3.54 (3H, s); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.7, 139.2, 138.7, 136.8, 132.9, 131.7, 129.3, 126.6, 124.3, 121.8, 120.3, 120.1, 115.6, 29.0; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 313.033 $C_{11}H_{12}N_2O_3S$ requires $(M-H)^-$ 313.0725]; **HPLC**: Retention time (system A): t_R= 9.92 min. Purity: >95%.

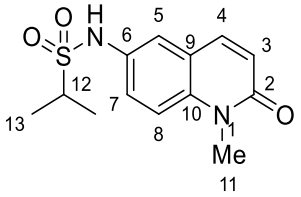


***N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)methanesulfonamide (16):** Prepared by general procedure E from **2** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane 3:7) to give the title compound (16 mg, 0.06 mmol, 59%) as a pale yellow solid: **mp** 239-242 °C (acetone-hexane); **IR** (neat) $\nu_{max:}$ 3129, 2925, 1635, 1580, 1565, 1422, 1319, 1148, 1119, 971, 815, 776, 735, 655, 542, 513, 467, 410 ; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 9.79 (1H, s, N*H*), 7.91 (1H, d, *J* = 9.3 Hz, *C*$_4$*H*), 7.58 - 7.52 (2H, m *C*$_5$*H and C*$_8$*H*), 7.46 (1H, dd, *J* = 9.6, 2.5 Hz, *C*$_7$*H*), 6.63 (1H, d, *J* = 9.3 Hz, *C*$_3$*H*), 3.60 (3H, s *C*$_{11}$*H*), 2.99 (3H, s, *C*$_{12}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.4 C_2, 138.6 C_4, 136.8 C_{10}, 132.4 C_6, 124.4 C_7, 121.8 C_3, 120.5 C_9, 119.7 C_5, 115.7 C_8, 39.5 (Assigned by HSQC) C_{12}, 28.3 C_{11}; **HRMS**: *m/z* NSI+ [Found $(M+H)^+$ 253.0648 $C_{11}H_{12}N_2O_3S$ requires $(M+H)^+$ 253.0569]; **HPLC**: Retention time (system A): t_R= 4.27 min. Purity: >95%.

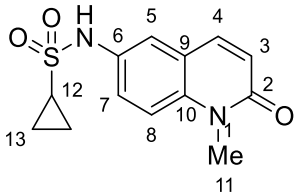


***N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)ethanesulfonamide (17)** Prepared by general procedure E from **2** (20 mg, 0.11 mmol). Purified by column chromatography (acetone-hexane 3:7)

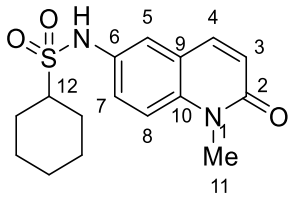
to give the title compound (21 mg, 0.07 mmol, 64%) as a pale yellow solid: **mp** 195-199 °C (acetone-hexane); **IR** (neat) $\nu_{max:}$ 3130, 2931, 1640, 1566, 1420, 1313, 1233, 1141, 977, 881, 818, 768, 527, 470 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 9.86 (1H, s, N*H*), 7.90 (1H, d, *J* = 9.3 Hz, *C*$_4$*H*), 7.56 - 7.51 (2H, m, *C*$_5$*H and C*$_8$*H*), 7.47 (1H, dd, *J* = 10.4, 2.0 Hz, *C*$_7$*H*), 6.62 (1H, d, *J* = 9.3 Hz, *C*$_3$*H*), 3.60 (3H, s, *C*$_{11}$*H*), 3.08 (2H, q, *J* = 7.3 Hz, *C*$_{12}$*H*), 1.21 (3H, t, *J* = 7.3 Hz, *C*$_{13}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.3 C_2, 138.9 C_4, 136.6 C_{10}, 132.5 C_6, 123.9 C_7, 121.8 C_3, 120.5 C_9, 119.2 C_5, 115.8 C_8, 44.9 C_{12}, 29.1 C_{11}, 8.0 C_{13}; **HRMS** *m/z* NSI+ [Found $(M+H)^+$ 267.0802 $C_{11}H_{12}N_2O_3S$ requires $(M+H)^+$ 267.0725]; **HPLC**: Retention time (system A): t_R= 3.94 min. Purity: >95%.



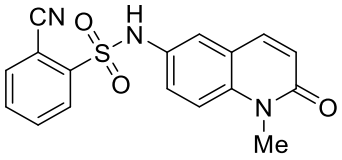
***N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)propane-2-sulfonamide (18)** Prepared by general procedure E from **2** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane 3:7) to give the title compound (13 mg, 0.05 mmol, 43%) as a brown solid: **mp** 191-193 °C (acetone-hexane); **IR** (neat) ν_{max} : 3084, 2933, 1622, 1572, 1486, 1306, 1259, 1139, 1121, 984, 886, 754, 695, 672, 586, 492, 473, 446, 411 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 9.85 (1H, s, N*H*), 7.90 (1H, d, *J*=9.6 Hz, *C*$_4$*H*), 7.57 - 7.45 (3H, m, *C*$_5$*H,C*$_7$*H and C*$_8$*H*), 6.61 (1H, d, *J* = 9.3 Hz, *C*$_3$*H*), 3.59 (3H, s *C*$_{11}$*H*), 3.22 (1H, quin, *J* = 6.8 Hz, *C*$_{12}$*H*), 1.25 (6H, d, *J* = 6.8 Hz, *C*$_{13}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.7 C_2, 138.8 C_4, 136.5 C_{10}, 132.7 C_6, 123.7 C_7, 121.8 C_3, 120.5 C_9, 118.9 C_5, 115.8 C_8, 51.2 C_{12}, 29.0 C_{11}, 16.1 C_{13}; **HRMS** *m/z* NSI+ [Found $(M+H)^+$ 281.0954 $C_{11}H_{12}N_2O_3S$ requires $(M+H)^+$ 281.0882]; **HPLC**: Retention time (system A): t_R= 5.53 min. Purity: >95%.



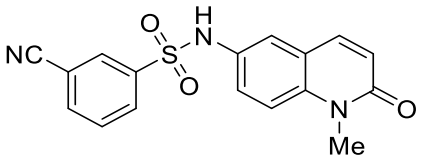
***N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)cyclopropanesulfonamide (19)** Prepared by general procedure E from **2** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane 3:7) to give the title compound (31 mg, 0.10 mmol, 97%) as a bright yellow solid: **mp**: 181-182 °C (acetone-hexane); **IR** (neat) $\nu_{max:}$ 3095, 2928, 1639, 1570, 1481, 1310, 1143, 978, 882, 809, 584, 525, 471 cm^{-1}; **^{1}H NMR** (500 MHz, DMSO-*d*$_6$): δ ppm 9.79 (1H, s, NH), 7.91 (1H, d, *J* = 9.5 Hz, *C*$_4$*H*), 7.57 (1H, s, *C*$_5$*H*), 7.54 (1H, d, *J* = 9.5 Hz, *C*$_8$*H*), 7.49 (1H, d, *J* = 8.5 Hz, *C*$_7$*H*), 6.63 (1H, d, *J* = 9.5 Hz, *C*$_3$*H*), 3.61 (3H, s, *C*$_{11}$*H*), 0.91 (4H, br. s); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.1 C_2, 138.8 C_4, 136.8 C_{10}, 132.4 C_6, 124.7 C_7, 121.8 C_3, 120.4 C_9, 120.2 C_5, 115.6 C_8 , 29.3 C_{12}, 29.1 C_{11}, 4.9 C_{13}; **HRMS** *m/z* ESI+ [Found $(M+H)^+$ 279.0800, $C_{13}H_{14}N_2O_3S$ requires $(M+H)^+$ 279.0825]; **HPLC**: Retention time (system A): t_R= 4.99 min. Purity: >95%.



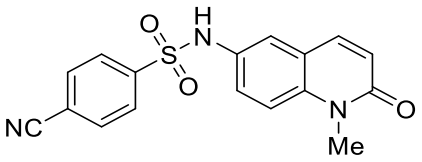
***N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)cyclohexanesulfonamide (20):** Prepared by general procedure E from **2** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane 3:7) to give the title compound (20 mg, 0.06 mmol, 59%) as a pale yellow solid: **mp** 169-172 °C (acetone-hexane); **IR** (neat) $\nu_{max:}$ 3098, 2933, 1640, 1620, 1572, 1483, 1332, 1308, 1258, 1142, 1121, 980, 882, 807, 758, 706, 604, 585, 526, 492, 472, 412 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 9.85 (1H, s), 7.90 (1H, d, *J* = 9.6 Hz), 7.56 - 7.45 (3H, m), 6.61 (1H, d, *J* = 9.6 Hz), 3.59 (3H, s), 3.04 – 2.82 (1H, m), 2.03 (2H, d, *J* = 11.1 Hz), 1.75 (2H, d, *J* = 12.6 Hz), 1.57 (1H, d, *J* = 11.6 Hz), 1.51 - 1.32 (2H, m), 1.26 - 1.02 (3H, m); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.7, 138.9, 136.4, 132.7, 123.6, 121.8, 120.5, 118.8, 115.8, 58.8, 29.0, 26.0, 24.7, 24.3; **HRMS** *m/z* ESI+ [Found $(M+H)^+$ 321.1267, $C_{16}H_{20}N_2O_3S$ requires $(M+H)^+$ 321.1195]; **HPLC**: Retention time (system A): t_R= 8.06 min. Purity: >95%.



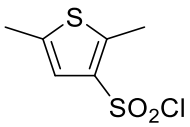
2-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (21): Prepared by general procedure E from **2** (100 mg, 0.55 mmol). Purified by column chromatography (ethyl acetate) to give the title compound (114 mg, 0.34 mmol, 61%) as an off white solid: **mp** 231 – 234 °C (ethyl acetate-hexane); **IR** (neat) ν_{max}: 3185, 2225, 1646, 1578, 1500, 1408, 1348, 1309, 1162, 1098, 956, 933, 908, 820, 797, 712, 681, 638, 590, 531, 472, 417; **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.19 (1H, t, *J*= 1.5 Hz), 8.15–8.07 (1H, m), 8.04–7.96 (1 H, m), 7.87 (1H, d, *J* = 9.5 Hz), 7.81–7.71 (1H, m), 7.50–7.40 (2H, m), 7.31 (1H, s), 6.60 (1H, d, *J* = 9.5 Hz), 3.54 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.0, 142.5, 138.9, 138.7, 131.9, 130.8, 128.0, 127.9, 127.6, 124.1, 122.7, 122.1, 119.6, 118.2, 116.1, 112.1, 29.4; **HRMS** *m/z* ESI+ [Found $(M+H)^+$ 340.0678 $C_{17}H_{13}N_3O_3S$ requires $(M+H)^+$ 340.0751]; **HPLC**: Retention time (system A): t_R= 9.28 min. Purity: >95%.



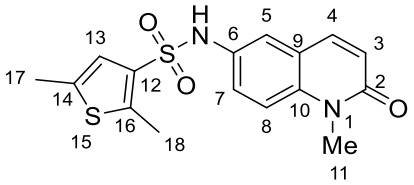
3-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (22): Prepared by general procedure E from **2** (100 mg, 0.55 mmol), purified by column chromatography (ethyl acetate) to give the title compound (88 mg, 0.26 mmol, 46%) as an off-white solid: **mp** 256-257 °C (ethyl acetate); **IR** (neat) ν_{max}:1637, 1565, 1503, 1462, 1436, 1416, 1336, 1239, 1204, 1156, 1124, 1087, 1062, 976, 934, 904, 873, 802, 715, 82, 625, 583, 560, 495, 472 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.53 (1H, br. s), 8.19 (1H, t, *J* = 1.5 Hz), 8.11 (1H, dt, *J* =7.8, 1.3 Hz), 8.03–7.97 (1H, m), 7.87 (1H, d, *J* = 9.5 Hz), 7.80 –7.72 (1H, m), 7.48–7.41 (2H, m), 7.33–7.26 (1H, m), 6.63 (d, *J* = 9.5 Hz,), 3.55 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.6, 140.3, 138.7, 137.2, 136.6, 131.1, 130.9, 130.8, 130.2, 124.7, 121.9, 120.8, 120.4, 117.4, 115.8, 112.5, 29.0; **HRMS** *m/z* ESI+ [Found $(M+H)^+$ 340.0678 $C_{17}H_{13}N_3O_3S$ requires $(M+H)^+$ 340.0751]; **HPLC**: Retention time (system A): t_R= 9.21 min. Purity: >95%.



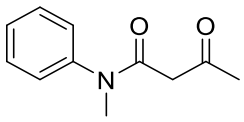
4-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (23): Prepared by general procedure E from **2** (100 mg, 0.55 mmol), purified by column chromatography (ethyl acetate) to give the title compound (77 mg, 0.23 mmol, 41%) as an off white solid: **mp**: >300 °C; **IR** (neat) ν_{max}: 3091, 2846, 1644, 1574, 1523, 1435, 1338, 1274, 1159, 1091, 952, 905, 871, 844, 663, 649, 584, 556, 496 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.59 (1H, br. s), 8.06–8.01 (2H, m), 7.91–7.84 (3H, m), 7.47–7.42 (2H, m), 7.30 (1H, d, *J* = 2.6 Hz), 6.60 (1H, d, *J* = 9.5 Hz), 3.55 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.8, 146.1, 143.2, 136.6, 133.3, 127.9, 124.5, 122.0, 120.1, 118.0, 117.2, 116.3, 116.0, 29.0; **HRMS** *m/z* ESI+ [Found $(M+H)^+$ 340.0755 $C_{17}H_{13}N_3O_3S$ requires $(M+H)^+$ 340.0751]; **HPLC**: Retention time (system A): t_R= 9.33 min. Purity: >95%.



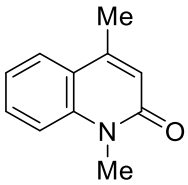
2,5-Dimethylthiophene-3-sulfonyl chloride (25) Dry DMF (211 mg, 2.9 mmol, 1.3 eq.) was added to flame dried flask and cooled to 0 °C. SO_2Cl_2 (392 mg, 2.9 mmol, 1.3 eq.) was added dropwise and the resultant yellow solution was stirred for 20 minutes at 0 °C. After this 2,5 dimethylthiophene (250 mg, 2.23 mmol, 1 eq.) was added and the solution heated to 97 °C for 2 hours before being allowed to cool back down to RT. The volatile solvents were removed under reduced pressure and the residue dissolved in DCM, washed with 5% $NaHCO_3$, dried over *anhydrous* $MgSO_4$, and filtered. The solvent was removed under reduced pressure to give the title compound (310 mg, 1.47 mmol, 66%) as a viscous black oil which was used without further purification.



2,5-Dimethyl-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)thiophene-3-sulfonamide (26): Prepared by general procedure E from **2** (100 mg, 0.55 mmol), purified by column chromatography (ethyl acetate: hexane 2:8-4:6) to give the title compound (59 mg, 0.17 mmol, 31%) as an off-white solid: **mp** 201-204 °C (ethyl acetate-hexane); **IR** (neat) ν_{max}: 3120, 2921, 2851, 1637, 1564, 1498, 1457, 1428, 1414, 1377, 1308, 1227, 1140, 1117, 1054, 1029, 939, 929, 898, 831, 805, 756, 698, 636, 587, 533, 499, 470, 424 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d_6*): δ ppm 10.23 (1H, br. s), 7.87 (1H, d, *J* = 9.6 Hz, *C_4H*), 7.47 (1H, d, *J* = 9.1 Hz, *C_8H*), 7.41 (1H, d, *J* = 2.5 Hz, *C_5H*), 7.32 (1H, dd, *J* = 9.1, 2.5 Hz, *C_7H*), 6.88 (1H, d, *J* = 1.3 Hz, *$C_{13}H$*), 6.60 (1H, d, *J* = 9.3 Hz, *C_3H*), 3.56 (3H, s, *$C_{11}H$*), 2.40 (3H, s, *$C_{18}H$*), 2.31 (3H, s, *$C_{17}H$*); **^{13}C NMR** (126 MHz, DMSO-*d_6*): δ ppm 160.7, 142.4, 138.7, 136.8, 136.3, 133.6, 131.7, 125.1, 124.2, 121.8, 120.3, 120.0, 115.6, 29.0, 14.5, 13.7**;** **HRMS** *m/z* ESI- [Found $(M-H)^-$ 347.0782 $C_{11}H_{12}N_2O_3S$ requires $(M-H)^-$ 347.0881].

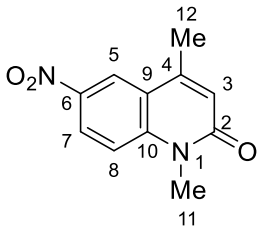


N-Methyl-3-oxo-N-phenylbutanamide (28) To a solution of *N*-methylaniline (3 g, 26.5 mmol, 1 eq.) in xylenes as added 2,2,6-Trimethyl-4*H*-1,3-dioxin-4-one (3.77 g, 26.5 mmol, 1 eq.) and the resulting solution was heated at reflux for 3 hours. After this time the solution was allowed to cool to RT and the solvent removed under reduced pressure. The crude oil was purified by column chromatography (ethyl acetates:hexane 4:1) to give the title compound (3.02 g, 15.6 mmol, 59%) as a dark red oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 14.26 (0.2 H, br. s), 7.45 - 7.39 (2H, m), 7.38 - 7.32 (1H, m), 7.22 - 7.16 (2H, m), 4.68 (0.2H, br. s), 3.35 - 3.24 (5H, m), 2.09 (2H, s), 1.79 (0.7H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 202.4, 166.4, 143.5, 129.9, 128.3, 127.2, 49.90 , 37.31, 30.33. The above data is in agreement with that previously reported in the literature. [109]

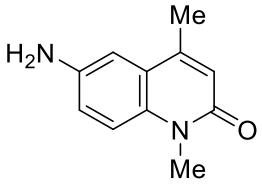


1,4-Dimethylquinolin-2(1*H*)-one (29) Concentrated sulfuric acid (10 mL) was cooled to 0 °C and **28** (3 g, 15.6 mmol 1 eq.) was added dropwise, the flask was fitted with a calcium chloride guard tube and then heated to 95 °C for 2 hours. After this time the solution was cooled to RT and then poured over crushed ice and stirred for 30 minutes. The resultant precipitate was filtered, washed with water and dried under vacuum to give the title compound (1.83 g, 10.5 mmol, 58%) as a purple solid: **mp** 132-134 °C (water); **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.73 (1H, dd, J = 8.1, 1.5

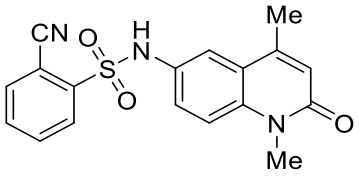
Hz), 7.60 (1H, ddd, *J* = 8.5, 7.1, 1.5 Hz), 7.40 (1H, d, *J* = 8.6 Hz), 7.31 - 7.25 (1H, m $CHCl_3$ overlap), 6.62 (1H, d, *J* = 1.0 Hz), 3.73 (3H, s), 2.49 (3H, d, *J* = 1.0 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.1, 146.4, 139.8, 130.4, 125.2, 121.9, 121.4, 121.2, 114.2, 29.2, 18.9. The above data is in agreement with that previously reported in the literature. [110]



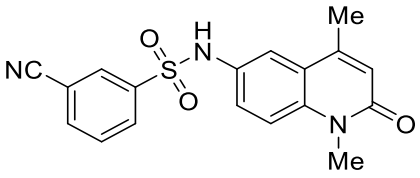
1,4-Dimethyl-6-nitroquinolin-2(1*H*)-one (30): Prepared according to general procedure C from **29** (1g, 5.78 mmol, 1 eq.) to give the title compound (1.15 g, 5.27 mmol, 91%) as a pale yellow solid : **^{1}H NMR**: (400 MHz, DMSO-*d*$_6$): δ ppm 8.42 (1H, d, *J* = 8.3 Hz, *C*$_7$*H*), 8.05 (1H, s, *C*$_5$*H*), 7.96 (1H, s, *C*$_3$*H*), 7.09 (1H, d, *J* = 8.6 Hz, *C*$_8$*H*), 3.60 (s, 3H, *C*$_{11}$*H*), 2.19 (s, 3H, *C*$_{12}$*H*). The above data is in agreement with that previously reported in the literature. [111]



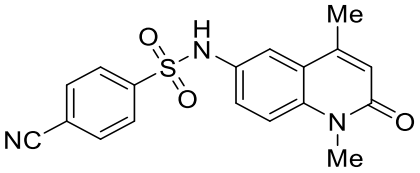
6-Amino-1,4-dimethylquinolin-2(1*H*)-one (31): Prepared according to general procedure D from **30** (900 mg, 4.12 mmol) to give the title compound (603 mg, 3.2 mmol, 78%) as a golden solid: **mp** 162-164 °C (DCM); **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.45 (1H, d, *J* = 8.1 Hz), 6.77 (1H, s), 6.65 (1H, s), 6.60 (1H, d, *J* = 8.2 Hz), 3.69–3.60 (5H, m), 2.10 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ ppm 161.6, 145.4, 141.1, 133.2, 122.4, 121.6, 119.1, 115.5, 109.7, 29.2, 19.0.



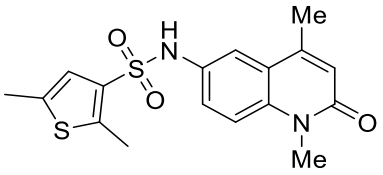
2-Cyano-*N*-(1,4-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (32): Prepared by general procedure E from **31** (100 mg, 0.53 mmol), purified by column chromatography (ethyl acetate) to give the title compound (39 mg, 0.09 mmol, 17%) as a white solid: **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 10.65 (1H, s), 8.91 (1H, d, *J* = 5.3 Hz), 8.02 - 7.96 (3H, m), 7.50 - 7.24 (3H, m), 6.54 (1H, s), 3.52 (3H, s), 2.31 (3H, s); **HRMS** *m/z* ESI- [Found $(M-H)^-$ 352.0740 $C_{18}H_{15}N_3O_3S$ requires $(M-H)^-$ 352.0756].



3-Cyano-*N*-(1,4-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (33): Prepared by general procedure E from **31** (100 mg, 0.53 mmol), purified by column chromatography (ethyl acetate) to give the title compound (46 mg, 0.13 mmol, 25%) as a yellow solid: **mp** 248 – 250 °C (ethyl acetate-hexane); **IR** (neat) ν_{max}: 2915, 2849, 1640, 1579, 1488, 1417, 1374, 1343, 1305, 1156, 1084, 970, 885, 795, 728, 678, 580, 498 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.53 (1H, s), 8.20 (1H, s), 8.11 (1H, d, *J* = 7.8 Hz), 8.01 (1H, d, *J* = 8.1 Hz), 7.77 (1H, t, *J* = 7.6 Hz), 7.46 - 7.40 (2H, m), 7.32 (1H, dd, *J* = 9.0, 2.4 Hz), 6.53 (1H, s), 3.52 (3H, s), 2.31 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.4, 145.5, 136.8, 136.5, 131.2, 130.8, 130.2, 124.4, 121.2, 120.9, 117.4, 117.3, 116.0, 112.5, 28.8, 18.1; **HRMS** *m/z* ESI- [Found $(M-H)^-$ 352.0749 $C_{18}H_{15}N_3O_3S$ requires $(M-H)^-$ 352.0756].

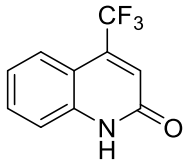


4-Cyano-*N*-(1,4-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (34): Prepared by general procedure E from **31** (100 mg, 0.53 mmol), purified by column chromatography (ethyl acetate) to give the title compound (73 mg, 0.21 mmol, 43%) as a white solid **mp** 281-282 °C (ethyl acetate); **IR** (neat) ν_{max}: 2915, 2849, 2776, 1637, 1616, 1564, 1482, 1339, 1309, 1283, 1159, 1091, 979, 869, 835, 815, 732, 702, 660, 628, 578, 546, 519, 499, 441 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.04 (2H, d, *J* = 8.3 Hz), 7.90 (2H, d, *J* = 8.3 Hz), 7.48–7.39 (2H, m), 7.31 (1H, dd, *J* = 9.0, 2.4 Hz), 6.54 (1H, s), 3.53 (3H, s), 2.31 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6) δ ppm 160.4, 145.5, 143.3, 136.9, 133.5, 127.5, 124.4, 121.2, 120.9, 117.5, 117.4, 116.0, 115.3, 28.8, 18.1; **HRMS** *m/z* ESI- [Found $(M-H)^-$ 352.0749 $C_{18}H_{15}N_3O_3S$ requires $(M-H)^-$ 352.0756]

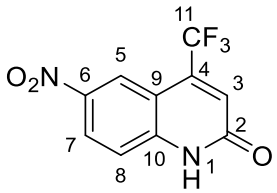


***N*-(1,4-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2,5-dimethylthiophene-3-sulfonamide (35) :** Prepared by general procedure E from **26** (100 mg, 0.53 mmol), purified by column chromatography (EtOAc:hexane ; 1:1) to give the title compound (88 mg, 0.24 mmol, 44%) as a white solid: **mp** 250-251 °C (decomposed); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.46 (1H, d, *J* = 9.1 Hz), 7.40 (1H, d, *J* = 1.8 Hz),7.33 (1H, dd, *J* = 9.0, 1.9 Hz), 6.90 (1H, s), 6.54 (1H, s), 3.54 (3H, s), 2.41

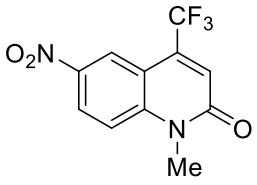
(3H, s), 2.36 - 2.28 (6H, app s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.4, 142.6, 138.7, 136.7, 136.8, 133.7, 131.8, 124.9, 124.2, 122.0, 120.8, 120.1, 115.5, 29.0, 28.1, 14.4, 13.7.



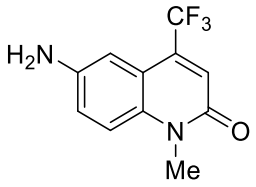
4-(Trifluoromethyl)quinolin-2(1*H*)-one (38) Prepared according to the procedure of van Oeveren *et al* from aniline (2.6 g, 27.9 mmol, 1 eq.) and 4,4,4 trifluoroacetoacetate (5.0 g, 27.1 mmol, 0.97 eq.) to give the title compound (2.2 g, 10.3 mmol, 38%) as a colourless solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 7.72 (1H, d, *J* = 8.2 Hz), 7.65 (1H, t, *J* = 7.6 Hz), 7.46 (1H, d, *J* = 7.9 Hz), 7.33 (1H, t, *J* = 7.6 Hz), 6.99 (1H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 159.9, 139.7, 136.4 (q, *J* = 31 Hz), 131.7, 124.1, 123.4, 122.9, 122.7 (q, *J* = 277 Hz) 121.8 (q, *J* = 5 Hz), 116.4, 112.6. The above data is in agreement with that previously reported in the literature. [72]



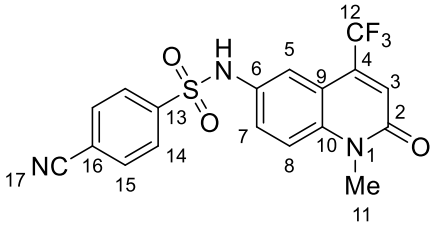
6-Nitro-4-(trifluoromethyl)quinolin-2(1*H*)-one (39) Prepared according to the procedure of van Oeveren *et al* from **38** (1.0 g, 4.69 mmol, 1 eq.) to give the title compound (874 mg, 3.38 mmol, 72 %) as yellow needles: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 12.85 (1H, br. s., *NH*), 8.47 (1H, dd, *J* = 9.0, 2.4 Hz, *C$_7$H*), 8.43 (1H, s, *C$_5$H*), 7.58 (1H, d, *J* = 9.1 Hz, *C$_8$H*), 7.21 (1H, s, *C$_3$H*); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.1, 143.9, 141.9, 136.0, 136.0 (q, *J* = 31 Hz), 126.2, 124.4 (q, *J* = 5 Hz), 122.3 (q, *J* = 277 Hz) 120.1, 117.5, 112.4. The above data is in agreement with that previously reported in the literature. [72]



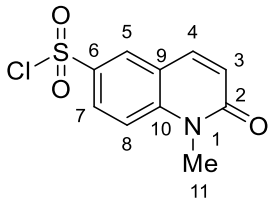
1-Methyl-6-nitro-4-(trifluoromethyl)quinolin-2(1*H*)-one (40) Prepared according to general procedure B from **39** (500 mg, 1.94 mmol). Crude product was crystallised from EtOH to give the title compound (488 mg, 1.78 mmol, 94%) as colourless needles: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.80 (1H, s), 8.54 (1H, dd, *J* = 9.3, 2.4 Hz), 7.59 (1H, d, *J* = 9.5 Hz), 7.26 (1H, s), 3.84 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 160.0, 144.2, 142.1, 136.9 (q, *J* = 33 Hz), 126.3, 123.3 (q, *J* = 5 Hz), 122.0 (q, *J* = 5 Hz), 120.1 (q, *J* = 278 Hz), 115.7, 114.8, 30.6. The above data is in agreement with that previously reported in the literature. [112]



6-Amino-1-methyl-4-(trifluoromethyl)quinolin-2(1*H*)-one (41) Prepared according to general procedure D from **40** (272mg, 1 mmol) to give the title compound (129 mg, 0.53 mmol, 53%) as a yellow solid: **mp** 171-172 °C (DCM); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.29 (1H, d, *J* = 9.1 Hz), 7.11 - 7.08 (2H, m), 7.06 (1H, dd, *J* = 8.8, 2.5 Hz), 3.81 (2H, br. s.), 3.72 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 159.8, 141.9, 136.0 (q, *J* = 31 Hz), 133.9, 122.3 (q, *J* = 275 Hz), 121.6 (q, *J* = 5 Hz), 120.4, 116.2, 116.0, 109.6 (q, *J* = 3 Hz), 29.9. The above data is in agreement with that previously reported in the literature. [112]

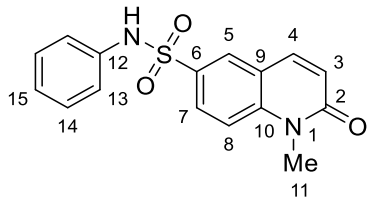


4-Cyano-*N*-(1-methyl-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-6-yl)benzenesulfonamide (42) Prepared according to general procedure E from **41** (24 mg, 0.1 mmol). Purified by column chromatography (acetone:hexane ; 3:7) to give the title compound (18 mg, 0.044 mmol, 44%) as colourless needles: **mp** 258-259 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.77 (1H, br. s. *NH*), 8.06 (2H, d, *J* = 8.3 Hz, $C_{15}H$), 7.88 (2H, d, *J* = 8.3 Hz, $C_{14}H$), 7.64 (1H, d, *J* = 9.1 Hz, C_8H), 7.48 (1H, dd, *J* = 9.1, 2.5 Hz, C_7H), 7.45 (1H, s, C_3H), 7.11 (1H, s, C_5H), 3.61 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 158.7, 142.9, 137.7, 134.4 (q, *J* = 31 Hz), 133.5, 131.8, 127.4, 125.8, 122.1 (q, *J* = 272 Hz), 122.1 (q, *J* = 4 Hz), 117.4, 117.3, 116.4, 115.5, 114.2, 29.7; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 406.0474 $C_{18}H_{12}N_3O_3SF_3$ requires $(M-H)^-$ 406.0473]; **HPLC**: Retention time (system A): t_R= 10.04 min. Purity: >95%.

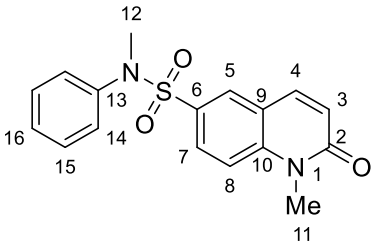


1-Methyl-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride (43): A solution of **1** (400 mg, 2.55 mmol, 1 eq.) in chlorosufonic acid (4 mL) was heated at 95 °C for 3 hours. After completion of the reaction the solution was cooled to 0 °C and added dropwise to ice water (~100 g, *Caution very violent reaction!*) The resulting precipitate was filtered, washed with water and dried under reduced pressure to give the title compound (503 mg, 1.96 mmol, 77 %) as an off-white solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 8.28 (1H, d, *J* = 2.0 Hz, C_5H), 8.19 (1H, dd, *J* = 9.1, 2.3 Hz, C_5H), 7.79

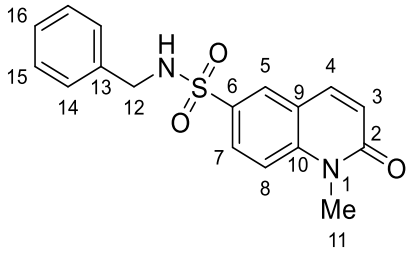
(1H, d, *J* = 9.6 Hz, *C*$_4$*H*), 7.56 (1H, d, *J* = 9.1 Hz, *C*$_8$*H*), 6.89 (1H, d, *J* = 9.6 Hz, *C*$_3$*H*), 3.80 (3H, s, *C*$_{11}$*H*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.8, 144.2, 138.3, 137.4, 128.4, 128.3, 124.3, 120.3, 115.4, 30.0.



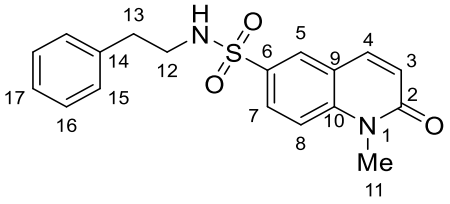
1-Methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-6-sulfonamide (44); From **43** (100 mg, 0.39 mmol, 1.1 eq.) and aniline (33 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (2:8 ethyl acetate:hexane) to give the title compound (82 mg, 0.27 mmol, 79%) as a colourless solid: **mp** 246-247 °C (ethyl acetate-hexane); **IR** (neat) ν_{max} : 3120, 3081, 3050, 2965, 2844, 1641, 1575, 1498, 1418, 1306, 1222, 1149, 1117, 1095, 1095, 936, 911, 762, 698, 690, 635, 594, 505, 476 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 10.31 (1H, s, *NH*), 8.17 (1H, d, *J* = 2.0 Hz, *C*$_5$*H*), 8.03 (1H, d, *J* = 9.6 Hz, *C*$_4$*H*), 7.90 (1H, dd, *J* = 9.0, 2.1 Hz, *C*$_7$*H*), 7.67 (1H, d, *J* = 9.1 Hz, *C*$_8$*H*), 7.25 - 7.19 (2H, m, *C*$_{14}$*H*), 7.14 - 7.09 (2H, m, *C*$_{13}$*H*), 7.01 (1H, tt, *J* = 7.3, 1.1 Hz, *C*$_{15}$*H*), 6.70 (1H, d, *J* = 9.6 Hz, *C*$_3$*H*), 3.60 (3H, s, *C*$_{11}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 161.0, 142.2, 138.9, 137.6, 132.5, 129.2, 128.1, 127.9, 124.1, 122.6, 120.0, 119.5, 115.9, 29.; **HRMS** *m/z* ESI- [Found (M-H)$^-$ 313.0635 $C_{16}H_{14}N_2O_3S$ requires (M-H)$^-$ 313.0647] ; **HPLC**: Retention time (system A): t_R= 8.29 min. Purity: >95%.



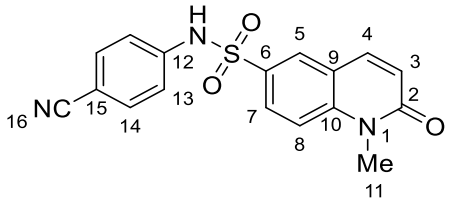
***N*,1-dimethyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-6-sulfonamide (45):** From **43** (100 mg, 0.39 mmol, 1.1 eq.) and *N*-methylaniline (36 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (1:9 - 2:8 ethyl acetate: hexane) to give the title compound (77 mg, 0.23 mmol, 71%) as a yellow solid: **mp** 149 -152 °C (ethyl acetate-hexane); **IR** (neat) ν_{max} : 1654, 1582, 1491, 1454, 1418, 1348, 1146, 1054, 860, 819, 758, 690, 636, 566, 507 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 8.07 (1H, d, *J* = 9.6 Hz, *C*$_4$*H*), 8.04 (1H, d, *J* = 2.0 Hz, *C*$_5$*H*), 7.70 - 7.64 (1H, m, *C*$_8$*H*), 7.58 (1H, dd, *J* = 9.0, 2.2 Hz, *C*$_7$*H*), 7.39 - 7.26 (3H, m, *C*$_{15}$*H/ C*$_{16}$*H*), 7.15 - 7.11 (2H, m, *C*$_{14}$*H*), 6.75 (1H, d, *J* = 9.5 Hz, *C*$_8$*H*), 3.64 (3H, s, *C*$_{11}$*H*), 3.17 (3H, s, *C*$_{12}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 161.1, 142.5, 141.1, 139.1, 129.1, 128.9, 128.8, 128.6, 127.3, 126.3, 122.6, 119.7, 115.5, 38.0, 29.4; **HRMS** *m/z* ESI- [Found (M+Cl)$^-$ 363.0567 $C_{17}H_{16}N_2O_3S$ requires (M+Cl)$^-$ 363.0570]; **HPLC**: Retention time (system A): t_R= 9.31 min. Purity: >95%.



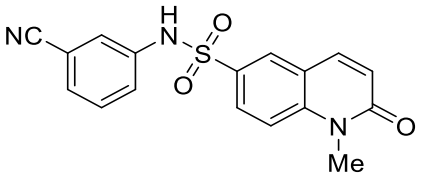
***N*-Benzyl-1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonamide (46)** From **43** (100 mg, 0.39 mmol, 1.1 eq.) and benzylamine (38 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (2:8 ethyl acetate: hexane) to give the title compound (100 mg, 0.30 mmol, 86%) as a colourless solid: **mp** 168 -170 °C (ethyl acetate-hexane); **IR** (neat) ν_{max}: 3091, 2854, 1642, 157, 1458, 1328,1154, 789, 732, 9, 640, 591, 506 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d_6*): δ ppm 8.23 - 8.15 (2H, m, *NH*/*C_5H*), 8.06 (1H, d, *J* = 9.6 Hz, *C_4H*), 7.96 (1H, dd, *J* = 9.0, 2.1 Hz, *C_7H*), 7.70 (1H, d, *J* = 9.1 Hz, *C_8H*), 7.25 - 7.16 (5H, m, *C_{14}H/ C_{15}H/ C_{16}H*), 6.74 (1H, d, *J* = 9.3 Hz, *C_3H*), 4.00 (2H, d, *J* = 6.3 Hz, *C_{12}H*), 3.65 (3H, s, *C_{11}H*); **^{13}C NMR** (126 MHz, DMSO-*d_6*): δ ppm 161.1, 141.9, 139.1, 137.3, 133.7, 128.2, 128.1, 127.6, 127.6, 127.1, 122.4, 119.6, 115.7, 46.1, 29.4**; HRMS** *m/z* ESI- [Found $(M-H)^-$ 327.0791 $C_{17}H_{16}N_2O_3S$ requires $(M-H)^-$ 327.0804]; **HPLC**: Retention time (system A): t_R= 8.26 min. Purity: >95%.



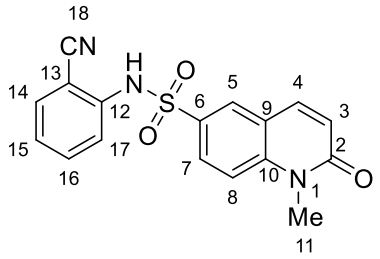
1-Methyl-2-oxo-*N*-phenethyl-1,2-dihydroquinoline-6-sulfonamide (47) From **43** (100 mg, 0.39 mmol, 1.1 eq.) and phenethylamine (42 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (2:8 ethyl acetate: hexane) to give the title compound (86 mg, 0.23 mmol, 72%) as a colourless solid: **mp** 159 -160 °C (ethyl acetate-hexane); **IR** (neat) ν_{max}: 3107, 2858, 1642, 1574, 1450, 1313, 1152, 1118, 1672, 896, 813, 754, 699, 636, 590, 510, 472, 435 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d_6*): δ ppm 8.17 (1H, d, *J* = 2.0 Hz, *C_5H*), 8.07 (1H, d, *J* = 9.6 Hz, *C_4H*), 7.93 (1H, dd, *J* = 9.0, 2.1 Hz, *C_7H*), 7.74 (1H, br. s. *NH*), 7.70 (1H, d, *J* = 9.1 Hz, *C_8H*), 7.28 - 7.11 (5H, m, *C_{15}H/ C_{16}H/ C_{17}H*), 6.73 (1H, d, *J* = 9.6 Hz, *C_3H*), 3.65 (3H, s, *C_{11}H*), 3.05 – 2.95 (2H, m, *C_{12}H*), 2.69 (2H, t, *J* = 7.5 Hz, *C_{13}H*); **^{13}C NMR** (126 MHz, DMSO-*d_6*): δ ppm 161.1, 141.9, 139.1, 138.6, 133.4, 128.6, 128.2, 128.1, 127.5, 126.3, 122.4, 119.6, 115.7, 44.1, 35.2, 29.4; **HRMS** *m/z* ESI- [Found $(M-H)^-$ 341.0954 $C_{18}H_{18}N_2O_3S$ requires $(M-H)^-$ 341.0960]; **HPLC**: Retention time (system A): t_R= 9.05 min. Purity: >95%.



***N*-(4-cyanophenyl)-1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonamide (48):** From **43** (100 mg, 0.39 mmol, 1.1 eq.) and 4-aminobenzonitrile (41 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (3:7 - 1:1 ethyl acetate: hexane) to give the title compound (47 mg, 0.14 mmol, 39%) as a colourless solid; **mp** 290-292 °C (decomposed); **IR** (neat) ν_{max} : 3128, 3050, 2937, 2876, 2221, 1646, 1575, 1503, 1349, 1303, 1159, 1098, 918, 836, 788, 690, 648, 01, 599, 512, 475; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 11.11 (1H, br. s. *NH*), 8.31 (1H, d, *J* = 2.0 Hz, *C*$_5$*H*), 8.08 (1H, d, *J* = 9.6 Hz, *C*$_4$*H*), 7.96 (1H, dd, *J* = 9.0, 2.1 Hz, *C*$_7$*H*), 7.73 – 7.67 (3H, m, *C*$_8$*H/ C*$_{13}$*H*), 7.27 (2H, d, *J* = 8.6 Hz, *C*$_{14}$*H*), 6.74 (1H, d, *J* = 9.3 Hz, *C*$_3$*H*), 3.61 (3H, s, *C*$_{11}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 161.0, 142.6, 142.1, 138.9, 133.7, 131.9, 128.0, 127.9, 122.8, 119.6, 118.7, 118.5, 116.2, 105.4, 29.4; **HRMS** *m/z* ESI- [Found (M-H)$^-$ 338.0591 $C_{17}H_{13}N_3O_3S$ requires (M-H)$^-$ 338.0599]; **HPLC**: Retention time (system A): t_R= 8.10 min. Purity: >95%.

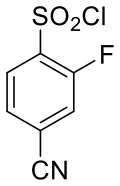


***N*-(3-cyanophenyl)-1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonamide (49):** From **43** (100 mg, 0.39 mmol, 1.1 eq.) and 3-aminobenzonitrile (41 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (3:7 - 1:1 ethyl acetate: hexane) to give the title compound (59 mg, 0.17 mmol, 50%) as a white solid: **mp** 234 -236 °C (ethyl acetate-hexane); **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 8.63 (1H, d, *J*=2.0 Hz), 8.47 (1H, dd, *J*=9.3, 2.8 Hz), 8.34 (1H, dd, *J*=8.1, 2.0 Hz), 8.28 (1H, d, *J*=2.5 Hz), 7.96-7.88 (m, 4H), 6.73 (1H, s), 3.66 (3H, s); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 161.1, 142.9, 137.8, 137.5, 132.1, 131.0, 128.1, 127.3, 127.1, 125.2, 123.8, 122.0, 119.9, 117.2, 116.5, 112.5, 29.3; **HRMS** *m/z* ESI- [Found (M-H)$^-$ 338.0593 $C_{17}H_{13}N_3O_3S$ requires (M-H)$^-$ 338.0599].

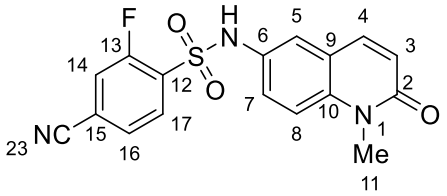


***N*-(2-cyanophenyl)-1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonamide (50):** From **43** (100 mg, 0.39 mmol, 1.1 eq.), 2-aminobenzonitrile (41 mg, 0.35 mmol, 1 eq.), 4-(Dimethylamino)pyridine (4 mg, 0.4 mmol, 0.1 eq.) and pyridine (31 mg, 32 µL, 1.1 eq.) were dissolved in DCM and heated at

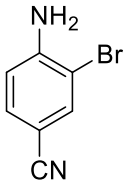
reflux overnight. The solvent was removed under reduced pressure and the residue purified by column chromatography (1:9 - 1:1 ethyl acetate: hexane) to give the title compound (18 mg, 0.05 mmol, 15%) as a brown solid: **IR** (neat) ν_{max} :1639, 1570, 1496, 1464, 1440, 1418, 1352, 1314, 1287, 1237, 1157, 1117, 1097, 1048, 935, 909, 832, 816, 787, 690, 648, 601, 599, 512, 475 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 10.62 (1H, br. s. *NH*), 8.14 (1H, d, *J* = 2.0 Hz, *C*$_5$*H*), 8.05 (1H, d, *J* = 9.6 Hz, *C*$_4$*H*), 7.90 (1H, dd, *J* = 9.1, 2.3 Hz, *C*$_7$*H*), 7.84 - 7.79 (1H, m, *C*$_{14}$*H*), 7.73 (1H, d, *J* = 9.1 Hz, *C*$_8$*H*), 7.59 (1H, td, *J* = 7.8, 1.5 Hz, *C*$_{16}$*H*), 7.40 (1H, td, *J* = 8.1, 0.8 Hz, *C*$_{15}$*H*), 7.07 (1H, d, *J* = 8.1 Hz, *C*$_{17}$*H*), 6.74 (1H, d, *J* = 9.3 Hz, *C*$_3$*H*), 3.64 (3H, s, *C*$_{11}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 161.1, 142.4, 139.1, 138.6, 134.1, 134.0, 132.5, 128.3, 128.0, 127.2, 126.3, 122.6, 119.6, 116.6, 115.9, 109.9, 29.4; **HRMS** *m/z* ESI- [Found $(M-H)^-$ 338.0591 $C_{17}H_{13}N_3O_3S$ requires $(M-H)^-$ 338.0599].



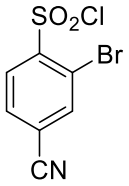
4-Cyano-2-fluorobenzenesulfonyl chloride (51c) Prepared by general procedure O from 4-cyano-2-fluoroaniline (2.0 g, 14.7 mmol) to give the title compound (2.66 g, 12.2 mmol, 83%) as an orange solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.16 (1H, dd, *J* = 8.0, 6.8 Hz), 7.75 - 7.66 (2H, m); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 158.2 (d, *J* = 264.9 Hz), 135.5 (d, *J* = 13.7 Hz), 130.4, 128.6 (d, *J* = 5.5 Hz), 121.9 (d, *J* = 23.8 Hz), 120.9 (d, *J* = 9.2 Hz), 115.2.



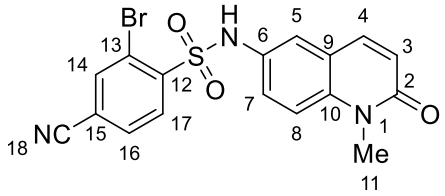
4-Cyano-2-fluoro-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (51d) Prepared according to general procedure E from **2** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 2:8-3-7) to give the title compound (31 mg, 0.09 mmol, 79%) as a pink solid: **mp** 282 -284 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-*d*$_6$): δ ppm 8.10 (1H, d, *J* = 10.1 Hz, *C*$_{14}$*H*), 7.95 (1H, t, *J* = 7.7 Hz, *C*$_{17}$*H*), 7.87 – 7.81 (2H, m, *C*$_{16}$*H/ C*$_4$*H*), 7.47 - 7.39 (2H, m, *C*$_8$*H/C*$_5$*H*), 7.33 (1H, dd, *J* = 9.1, 2.5 Hz, *C*$_7$*H*), 6.58 (1H, d, *J* = 9.5 Hz, *C*$_3$*H*), 3.55 (3H, s, *C*$_{11}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.7, 157.5 (d, *J* = 263.0 Hz), 138.7, 137.2, 131.6 (d, *J* = 14.7 Hz), 131.3, 130.5, 129.4 (d, *J* = 4.6 Hz), 124.6, 121.9, 121.6 (d, *J* = 25.7 Hz), 120.6, 120.4, 117.4 (d, *J* = 10.1 Hz), 116.4, 115.8, 29.0; **HRMS**: *m/z* FTMS- [Found $(M-H)^-$ 356.0508 $C_{17}H_{12}FN_2O_3S$ requires $(M-H)^-$ 356.0505]; **HPLC**: Retention time (system A): t_R= 7.92 min. Purity: 94%.



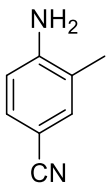
4-Amino-3-bromobenzonitrile (52a) Prepared according to the procedure of Suh *et al* from 4-cyanoaniline (250 mg, 1.27 mmol) to give the title compound (158 mg, 0.80 mmol, 63%) as an off-white solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.71 (1H, d, *J* = 1.6 Hz), 7.39 (1H, dd, *J* = 8.4, 1.7 Hz), 6.76 (1H, d, *J* = 8.2 Hz), 4.67 (2H, br. s.). The above data is in agreement with that reported previously in the literature. [113]



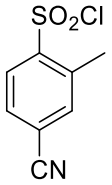
4-Cyano-2-bromobenzenesulfonyl chloride (52c) Prepared by general procedure O from **52a** (150 g, 0.76 mmol) to give the title compound (114 mg, 0.41 mmol, 54%) as a brown solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.12 (1H, d, *J* = 1.6 Hz), 8.03 (1H, d, *J* = 7.9 Hz), 7.83 (1H, dd, *J* = 8.0, 1.6 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 151.1, 137.1, 131.1, 129.5, 120.2, 117.2, 112.6.



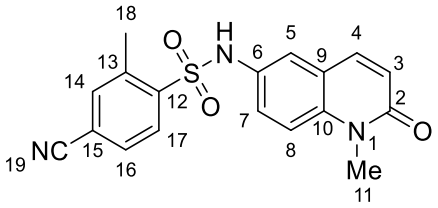
2-Bromo-4-cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (52d) Prepared according to general procedure E from **2** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 2:8-3:7) to give the title compound (33 mg, 0.08 mmol, 71%) as a colourless solid: **mp** 262 -263 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.65 (1H, s, *NH*), 8.16 (1H, d, *J* = 8.3 Hz, $C_{17}H$), 8.03 (1H, d, *J* = 1.5 Hz, $C_{14}H$), 7.88 (1H, d, *J* = 9.6 Hz, C_4H), 7.79 (1H, dd, *J* = 8.2, 1.6 Hz, $C_{16}H$), 7.51 - 7.41 (2H, m, C_8H/ C_5H), 7.31 (1H, dd, *J* = 8.8, 2.5 Hz, C_7H), 6.61 (1H, d, *J* = 9.6 Hz, C_3H), 3.56 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.7, 144.5, 138.7, 137.4, 136.5, 135.9, 130.5, 127.5, 125.8, 125.0, 122.0, 121.2, 120.5, 116.1, 115.9, 114.9, 29; **HRMS**: *m/z* NSI+ [Found $(M+H)^+$ 417.9856 $C_{17}H_{12}BrN_3O_3S$ requires $(M+H)^+$ 417.7983]; **HPLC**: Retention time (system A): t_R= 9.19 min. Purity: >95%.



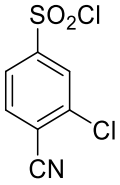
4-Amino-3-methylbenzonitrile (53a) Prepared by general procedure N from 4-nitro-3-methylbenzonitrile (1.0 g, 6.0 mmol). Crystallized (Et_2O:Hexane ; 1:2) to give the title compound (615 mg, 4.6 mmol, 77%) as colourless needles: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.38 – 7.31 (2H, m), 6.66 (1H, d, *J* = 8.8 Hz), 4.10 (2H, br. s.), 2.18 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 148.7, 134.2, 131.6, 122.1, 120.3, 114.2, 100.7, 17.0. The above data is in agreement with that previously reported in the literature. [114]



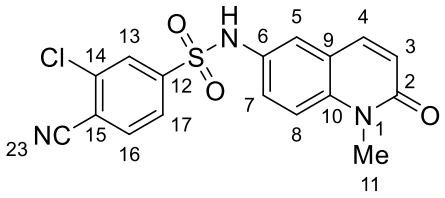
4-Cyano-2-methylbenzenesulfonyl chloride (53c) Prepared by general procedure O from **53a** (500 mg, 3.78 mmol) to give a crude solid of the crude title compound (128 mg) as an orange solid which was used without further purification.



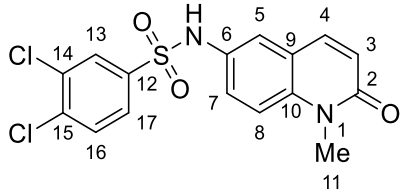
4-Cyano-2-methyl-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (53d) Prepared according to general procedure E from **2** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 2:8-3-7) to give the title compound (5 mg, 0.01 mmol, 12%) as a yellow solid: **mp** 271 -273 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 7.96 (1H, d, *J* = 8.3 Hz, *C*$_{17}$*H*), 7.90 (1H, s, *C*$_{14}$*H*), 7.90 – 7.70 (2H, m, *C*$_{16}$*H*/*C*$_4$*H*), 7.47 - 7.19 (3H, m, *C*$_8$*H*/*C*$_5$*H*/*C*$_7$*H*), 6.57 (1H, d, *J* = 9.1 Hz, *C*$_3$*H*), 3.53 (3H, s, *C*$_{11}$*H*), 2.64 (3H, s, *C*$_{18}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.7, 138.7, 138.3, 136.1, 130.2, 129.9, 124.0, 121.9, 120.4, 119.8, 117.5, 115.8, 115.2, 29.0, 19.5; **HRMS**: *m/z* FTMS- [Found $(M-H)^-$ 352.0764 $C_{18}H_{15}N_3O_3S$ requires $(M-H)^-$ 352.0756]; **HPLC**: Retention time (system A): t_R= 8.48 min. Purity: >95%.



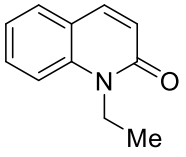
4-Cyano-3-chlorobenzenesulfonyl chloride (54c) Prepared by general procedure O from 3-chloro-4-cyanoaniline (500 mg, 3.29 mmol) to give the title compound (535 mg, 2.27 mmol, 69%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.19 (1H, d, *J* = 7.9 Hz), 7.96 – 8.06 (m, 2H). The above data is in agreement with that previously reported in the literature. [78]



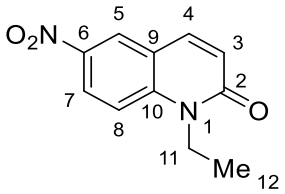
3-Chloro-4-cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (54d)
Prepared according to general procedure E from **2** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 2:8-3:7) to give the title compound (30 mg, 0.08 mmol, 73%) as a colourless solid: **mp** 245 °C (decomposed); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.16 (1H, d, *J* = 8.1 Hz, $C_{16}H$), 8.03 (1H, d, *J* = 1.5 Hz, $C_{13}H$), 7.88 (1H, d, *J* = 9.6 Hz, C_4H), 7.79 (1H, dd, *J* = 8.2, 1.6 Hz, $C_{17}H$), 7.49 - 7.42 (2H, m, C_8H/C_5H), 7.31 (1H, dd, *J* = 9.1, 2.5 Hz, C_7H), 6.61 (1H, d, *J* = 9.3 Hz, C_3H), 3.56 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.7, 144.6, 138.7, 137.4, 136.5, 135.9, 130.5, 127.5, 125.8, 125.0, 121.9, 121.2, 120.5, 116.1, 115.9, 115.0, 29.0; **HRMS**: *m/z* FTMS- [Found $(M-H)^-$ 372.0219 $C_{17}H_{12}ClN_3O_3S$ requires $(M-H)^-$ 372.0221]; **HPLC**: Retention time (system A): t_R= 9.19 min. Purity: >95%.



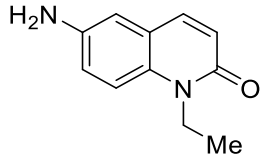
3,4-Dichloro-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (55d)
Prepared according to general procedure E from **2** (20 mg, 0.11 mmol). The crude solution was added to H_2O (10 mL) and filtered. Purification of the crude solid by crystallisation (MeOH) gave the title compound (38 mg, 0.10 mmol, 91%) as a colourless solid: **mp** 230 - 232 °C (methanol); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.62–10.32 (1H, br. s, *NH*), 7.95 (1H, d, *J* = 2.2 Hz, $C_{13}H$), 7.88 (1H, d, *J* = 9.5 Hz, C_4H), 7.83 (1H, d, *J* = 8.5 Hz, $C_{16}H$), 7.65 (1H, dd, *J* = 8.5, 2.2 Hz, $C_{17}H$), 7.48 – 7.42 (2H, m, C_8H/C_5H), 7.31 (1H, dd, *J* = 9.0, 2.6 Hz, C_7H), 6.60 (1H, d, *J* = 9.5 Hz, C_3H), 3.55 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.7, 139.5, 138.7, 137.3, 136.1, 132.2, 131.7, 130.9, 128.4, 126.8, 124.8, 121.9, 120.9, 120.4, 115.8, 29.0; **HRMS**: *m/z* FTMS- [Found $(M-H)^-$ 380.9871 $C_{17}H_{12}Cl_2N_2O_3S$ requires $(M-H)^-$ 380.9868]; **HPLC**: Retention time (system A): t_R= 10.34 min. Purity: >95%.



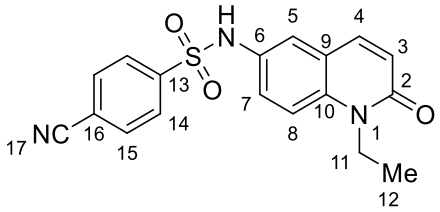
1-Ethylquinolin-2(1*H*)-one (56) Prepared by general procedure B from **14** (1.0 g, 6.8 mmol) Purification by column chromatography (1:1 ethyl acetate:hexane) gave the title compound (759 mg, 4.39 mmol, 66%) as a colourless oil. **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.90 (1H, d, *J* = 9.6 Hz), 7.73 (1H, d, *J* = 7.6 Hz), 7.66–7.56 (2H, m), 7.27 (1H, t, *J* = 7.8 Hz), 6.61 (1H, d, *J* = 9.6 Hz), 4.29 (2H, q, *J* = 7.1 Hz), 1.22 (3H, t, *J* = 7.1 Hz). The above data is in agreement with that previously reported in the literature. [115]



1-Ethyl-6-nitroquinolin-2(1*H*)-one (57) To a suspension of **56** (759 mg, 4.39 mmol, 1.0 eq.) in concentrated H_2SO_4 (5 mL) at –5 °C was added KNO_3 (444 mg, 4.39 mmol, 1.0 eq.) portionwise and the resulting yellow solution was stirred at this temperature for 1 hour before being allowed to warm to room temperature. The solution was poured over crushed ice, stirred for 10 minutes and the precipitate collected by filtration and dried under vacuum to give the title compound (804 mg, 3.68 mmol, 84%) as a pale yellow solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.71 (1H, d, *J* = 2.8 Hz, *C*$_5$*H*), 8.37 (1H, dd, *J* = 9.4, 2.5 Hz, *C*$_7$*H*), 8.13 (1H, d, *J* = 9.6 Hz, *C*$_4$*H*), 7.77 (1H, d, *J* = 9.6 Hz, *C*$_8$*H*), 6.77 (1H, d, *J* = 9.6 Hz, *C*$_3$*H*), 4.30 (2H, q, *J* = 7.1 Hz, *C*$_{11}$*H*), 1.22 (3H, t, *J* = 7.1 Hz, *C*$_{12}$*H*); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.7, 142.8, 141.3, 139.3, 125.1, 124.9, 122.9, 120.0, 115.5, 37.3, 12.6.

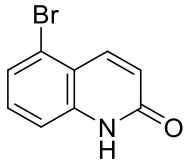


6-Amino-1-ethylquinolin-2(1*H*)-one (58) Prepared according to general procedure D from **57** (500 mg, 2.29 mmol, 1.0 eq.) to give the title compound (428 mg, 2.29 mmol, quant.) as a bright yellow solid: **mp** 158 - 159 °C (DCM); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.67 (1H, s), 7.30 (1H, d, *J*= 9.1 Hz), 6.96 (1H, dd, *J* = 9.1, 2.5 Hz), 6.80 (1H, d, *J* = 2.5 Hz), 6.47 (1H, d, *J* = 9.4 Hz), 5.08 (2H, br. s), 4.20 (2H, q, *J* = 7.1 Hz), 1.18 (3H, t, *J* = 7.1 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.3, 141.1, 138.1, 132.3, 122.3, 122.0, 119.5, 115.1, 112.9, 37.0, 12.8.

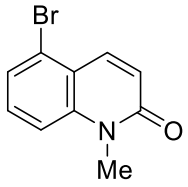


4-Cyano-*N*-(1-ethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (59) Prepared by general procedure E from **58** (50 mg, 0.27 mmol). Purified by column chromatography (acetone:hexane ; 2:8-3:7) to give the title compound (43 mg, 0.12 mmol, 45 %) as an off-white

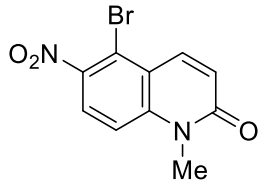
solid: **mp** 239 - 240 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.58 (1H, br. s, *NH*), 8.04 (2H, d, *J* = 8.3 Hz, $C_{15}H$), 7.90 (2H, d, *J* = 8.3 Hz, $C_{14}H$), 7.85 (1H, d, *J* = 9.6 Hz, C_4H), 7.50 (1H, d, *J* = 9.1 Hz, C_8H), 7.44 (1H, d, *J* = 2.3 Hz, C_5H), 7.29 (1H, dd, *J* = 9.0, 2.4 Hz, C_7H), 6.58 (1H, d, *J* = 9.6 Hz, C_3H), 4.20 (2H, q, *J* = 6.9 Hz, $C_{11}H$), 1.16 (3H, t, *J* = 6.9 Hz, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.3, 143.3, 138.8, 136.1, 133.5, 130.7, 127.4, 124.8, 121.9, 120.9, 120.7, 117.5, 115.6, 115.4 , 36.6, 12.5; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 352.0768 $C_{18}H_{15}N_3O_3S$ requires $(M-H)^-$ 352.0756]; **HPLC**: Retention time (system A): t_R= 8.39 min. Purity: >95%.



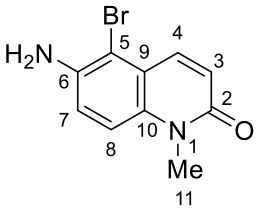
5-Bromoquinolin-2(1*H*)-one (61) Prepared according to general procedure A from 3-bromoquinoline (2.5 g, 12.0 mmol) to give the title compound (2.37 g, 10.60 mmol, 88%) as a colourless solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 11.98 (1H, br. s), 8.03 (1H, d, *J* = 9.9 Hz), 7.49–7.46 (1H, m), 7.42 (1H, t, *J* = 7.9 Hz), 7.34 (1H, d, *J* = 8.6 Hz), 6.64 (1H, d, *J* = 9.9 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.5, 140.2, 138.1, 131.4, 125.6, 123.7, 121.7, 117.8, 114.8. The above data is in agreement with that previously reported in the literature. [116]



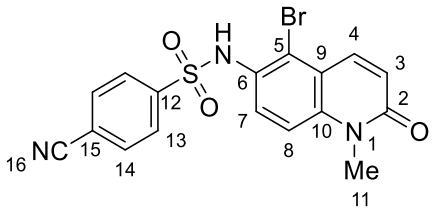
5-Bromo-1-methylquinolin-2(1H)-one (62) Prepared according to general procedure B from **61** (1.25 g, 5.58 mmol) to give the title compound (625 mg, 2.62 mmol, 47%) as a colourless solid: **^{1}H NMR** ($CDCl_3$): δ ppm 8.39 (1H, d, *J* = 8.0 Hz), 7.84 (1H, d, *J* = 8.1 Hz), 7.30 (1H, dd, *J* = 8.1, 7.9 Hz), 7.13 (1H, d, *J* = 7.9 Hz), 6.81 (1H, d, *J* = 7.9 Hz), 3.58 (3H, s). The above data is in agreement with that previously reported in the literature. [117]



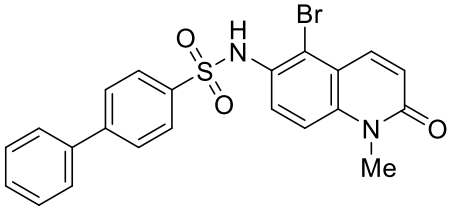
5-Bromo-1-methyl-6-nitroquinolin-2(1*H*)-one (63) Prepared according to general procedure C from **62** (500 mg, 2.1 mmol) to give the title compound (315 mg, 1.11 mmol, 53%) as a yellow solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.26 (1H, d, *J* = 10.1 Hz), 8.21 (1H, d, *J* = 9.4 Hz), 7.77 (1H, d, *J* = 9.4 Hz), 6.91 (1H, d, *J* = 9.9 Hz), 3.68 (3H, s).



6-Amino-5-bromo-1-methylquinolin-2(1*H*)-one (64) Prepared according to general procedure D from **63** (300 mg, 1.06 mmol) to give the title compound (185 mg, 0.73 mmol, 69%) as a bright yellow solid: **mp** 175 – 178 °C (DCM); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.98 (1H, d, *J* = 9.9 Hz, C_4H), 7.39 (1H, d, *J* = 9.1 Hz, C_8H), 7.18 (1H, d, *J* = 9.1 Hz, C_7H), 6.66 (1H, d, *J* = 9.9 Hz, C_4H), 5.39 (2H, br. S, *NH*), 3.58 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.2, 139.7, 137.1, 134.1, 123.1, 120.3, 119.0, 114.4, 108.0, 29.8. The above data is in agreement with that previously reported in the literature. [79]

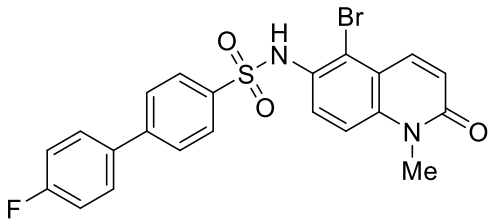


***N*-(5-bromo-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-4-cyanobenzenesulfonamide (65)** Prepared by general procedure E from **63** (20 mg, 0.08 mmol). Purified by column chromatography (acetone:hexane ; 6:4) to give the title compound (10 mg, 0.02 mmol, 28%) as a yellow solid: **mp** 240 - 242 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.46 (1H, br. s, *NH*), 8.11–7.96 (3H, m, $C_{14}H/C_4H$), 7.84 (2H, d, *J* = 8.3 Hz, $C_{13}H$), 7.57 (1H, d, *J* = 9.4 Hz, C_8H), 7.40 (1H, d, *J* = 9.1 Hz, C_7H), 6.73 (1H, d, *J* = 9.9 Hz, C_3H), 3.61 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.3, 144.6, 139.6, 137.6, 133.5, 130.8, 129.0, 127.4, 123.3, 122.4, 119.4, 117.7, 115.3, 115.2, 29.6; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 415.9703, $C_{19}H_{17}N_3O_4SBr$ requires $(M-H)^-$ 415.9705]; **HPLC**: Retention time (system A): t_R= 8.45 min. Purity: >95%.

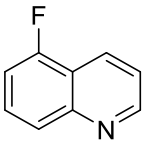


***N*-(5-bromo-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-[1,1'-biphenyl]-4-sulfonamide (66)** Prepared by general procedure E from **63** (20 mg, 0.08 mmol). Purified by column chromatography (EtOAc:hexane ; 1:1) to give the title compound (18 mg, 0.04 mmol, 47%) as a colourless solid: **mp** 227 - 228 °C (ethyl acetate-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.14 (1H, s), 8.04 (1H, d, *J* = 9.9 Hz), 7.88 (2H, d, *J* = 8.6 Hz), 7.81 - 7.71 (4H, m), 7.57 (1H, d, *J* = 9.3 Hz), 7.50 (2H, t, *J* = 7.1 Hz), 7.44 (2H, t, *J* = 9.1 Hz), 6.73 (1H, d, *J* = 9.9 Hz), 3.61 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.3, 144.2, 139.4, 139.2, 138.2, 137.6, 130.2, 129.6 , 129.1, 128.6, 127.4, 127.3, 127.0, 123.2,

122.0, 119.4, 115.2, 29.5; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 467.0070, $C_{22}H_{17}N_2O_3SBr$ requires $(M-H)^-$ 467.0065]; **HPLC**: Retention time (system A): t_R= 11.78 min. Purity: >95%.

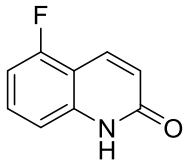


***N*-(5-bromo-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-4'-fluoro-[1,1'-biphenyl]-4-sulfonamide (67)** Prepared by general procedure E from **63** (20 mg, 0.08 mmol). Purified by column chromatography (EtOAc:hexane ; 1:1) to give the title compound (21 mg, 0.04 mmol, 54%) as a colourless solid: **mp** 219 - 220 °C (ethyl acetate-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.14 (1H, s), 8.05 (1H, d, *J* = 9.9 Hz), 7.86 (2H, d, *J* = 8.3 Hz), 7.83 - 7.74 (4H, m), 7.57 (1H, d, *J* = 9.1 Hz), 7.43 (1H, d, *J* = 9.3 Hz), 7.34 (2H, t, *J* = 8.8 Hz), 6.73 (1H, d, *J* = 9.9 Hz), 3.61 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 163.4, 161.5, 160.3, 143.1, 139.3 (d, *J*=21.1 Hz), 137.6, 134.7 (d, *J* = 2.7 Hz), 130.2, 129.6, 129.2 (d, *J*=8.2 Hz), 127.3 (d, *J*=15.6 Hz), 123.2, 122.0, 119.3, 116.1, 115.9, 115.1, 29.6; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 484.9977, $C_{22}H_{16}N_2O_3SF$ requires $(M-H)^-$ 484.9981]; **HPLC**: Retention time (system A): t_R= 11.78 min. Purity: >95%.

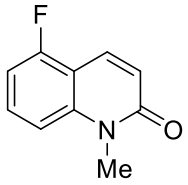


5-Fluoroquinoline (69) To a suspension of 5-aminoquinoline (2.50 g, 17.36 mmol, 1 eq.) in 48percent HBF_4 (20 mL) at 0°C was added portionwise sodium nitrite (1.32 g, 19.09 mmol, 1.1 eq.). The resulting solution was stirred for 1 hour at 0 °C and then poured into EtOAc:Et_2O (1:1, 50 mL). The diazonium tetrafluoroborate salt was filtered, washed with Et_2O (100 mL) and dried under vacuum.

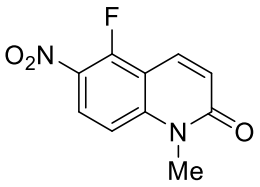
Xylene (80 mL) was heated to reflux and the tetrafluoroborate salt was added portionwise and then stirred at reflux for 2 hours before being cooled to RT. The xylene was decanted off and the residue was dissolved in HCl (1M, 50 mL). The solution was neutralized with $NaHCO_3$ and extracted with EtOAc (3 × 50 mL). The extracts were combined, dried over *anhydrous* Na_2SO_4, filtered and the solvent removed under reduced pressure. The residue was purified by column chromatography (EtOAc:Hexane 1:99 – 2:98) to give the title compound (970 mg, 6.50 mmol, 38%) as a pink oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 8.99 (1H, dd, *J* = 4.2, 1.6 Hz), 8.47 (1H, d, *J* = 8.3 Hz), 7.95 (1H, d, *J* = 8.6 Hz), 7.67 (1H, td, *J* = 8.2, 6.1 Hz), 7.49 (1H, dd, *J* = 8.3, 4.3 Hz), 7.28 - 7.21 (1H, m); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 158.1 (d, *J* = 252.4 Hz), 151.2, 149.0, 129.7, 129.0 (d, *J* = 9.0 Hz), 124.8 (d, *J* = 5.1 Hz), 121.3 (d, *J* = 3 Hz), 119.0, 110.0 (d, *J* = 19.2 Hz). The above data is in agreement with that previously reported in the literature. [118]



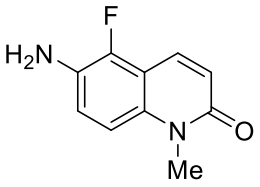
5-Fluoroquinolin-2(1*H*)-one (70) Prepared by general procedure A from **69** (950 mg, 6.46 mmol) to give the title compound (864 mg, 5.30 mmol, 82%) as a pale pink solid: **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 12.04 – 11.95 (1H, br. s.), 7.99 (1H, d, *J* = 9.6 Hz), 7.51 (1H, d, *J* = 6.3 Hz), 7.14 (1H, d, *J* = 8.3 Hz), 7.04 - 6.95 (1H, m), 6.57 (1H, d, *J* = 9.9 Hz).



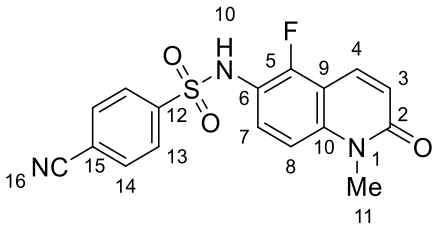
5-Fluoro-1-methylquinolin-2(1*H*)-one (71) Prepared by general procedure B from **70** (700 mg, 4.30 mg) to give the title compound (534 mg, 3.00 mmol, 70%) as a pale pink solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.01 (1H, d, *J* = 9.6 Hz), 7.65 (1H, td, *J* = 8.4, 6.4 Hz), 7.39 (1H, d, *J*=8.6 Hz), 7.13 (1 H, ddd, *J*=9.7, 8.2, 0.8 Hz), 6.69 (1 H, d, *J*=9.9 Hz), 3.63 (3 H, s).



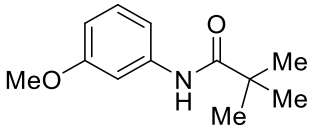
5-Fluoro-1-methyl-6-nitroquinolin-2(1*H*)-one (72) Prepared by general procedure C from **71** (400 mg, 2.26 mmol). The crude solid was crystallised (Acetone) to give the title compound (217 mg, 0.97 mmol, 43 %) as a yellow solid that was used without further purification.



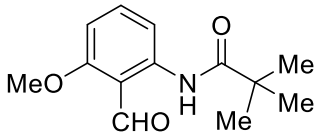
5-Fluoro-1-methyl-6-aminoquinolin-2(1*H*)-one (73) Prepared by general procedure D from **72** (100 mg, 0.45 mmol) to give the title compound (45 mg, 0.24 mmol, 54 %) as a bright yellow solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.21 (1H, dd, *J* = 9.0, 5.8 Hz), 8.12 (1H, d, *J* = 9.8 Hz), 7.34 (1H, t, *J* = 8.7 Hz), 6.85 (1H, d, *J*=9.5 Hz), 3.50 (2H, br. s.), 3.29 (3H, s).



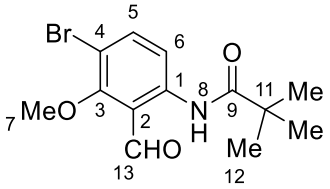
4-Cyano-*N*-(5-fluoro-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (74) Prepared by general procedure E from **73** (30 mg, 0.16 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 3:7) to give the title compound (44 mg, 0.13 mmol, 80%): **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.54 (1H, br. s, *NH*), 8.06 (2H, d, *J* = 8.6 Hz, $C_{14}H$), 7.92 - 7.82 (3H, m, $C_{13}H/C_4H$), 7.45 - 7.37 (1H, m C_7H), 7.33 (1H, d, *J* = 9.6 Hz, C_8H), 6.66 (1H, d, *J* = 9.6 Hz, C_3H), 3.58 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.2, 154.1 (d, *J* = 244.6 Hz), 150.9, 144.7 (d, *J* = 13.4 Hz), 135.5, 135.4, 128.9 (d, *J* = 18 Hz), 126.2, 122.7, 119.2 , 117.0, 116.0, 102.6, 102.2, 29.2; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 356.0496 $C_{17}H_{12}N_3O_3SF_3$ requires $(M-H)^-$ 356.0505].



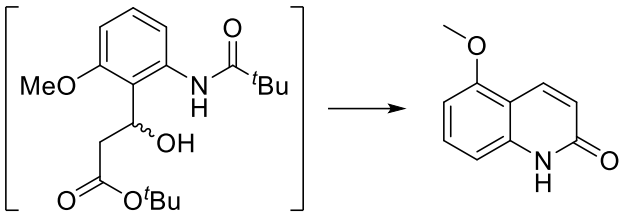
***N*-(3-methoxyphenyl)pivalamide (76)** 3-methoxyaniline (5.0 g, 40.65 mmol, 1 eq.) and pyridine (3.5 g 44.72 mmol, 1.1 eq.) were dissolved in DCM (50 mL) and cooled down to 0 °C. Pivaloyl chloride (5.4 g, 44.72 mmol, 1.1 eq.) was added dropwise with rapid stirring, the solution was allowed to warm to RT and then stirred overnight. HCl (1M, 50 mL) was added and the aqueous phase extracted with DCM (3 × 100 mL), the organic fractions were pooled, dried over *anhydrous* $MgSO_4$ and concentrated under reduced pressure to give the title compound (8.4 g, 40.60 mmol, quant.) as an off-white solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.42 (1H, t, *J* = 1.9 Hz), 7.34 (1H, br. s.), 7.22 (1H,app. t, *J* = 8.2 Hz), 6.96 (1H, d, *J* = 7.9 Hz), 6.68 (1H, dd, *J* = 8.2, 2.2 Hz), 3.83 (3H, s), 1.34 (9H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 176.6, 160.2, 139.3, 129.6, 111.8, 110.4, 105.3, 55.3, 39.7, 27.6. The above data is in agreement with that previously reported in the literature. [82]



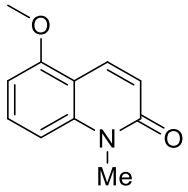
***N*-(2-formyl-3-methoxyphenyl)pivalamide (77)** Prepared according to the procedure of Bertani *et al* [83] starting from **76** (5.0 g, 24.15 mmol). Purification by column chromatography (EtOAc:hexane ; 1:19 – 3:17) gave the title compound (4.14 g, 17.63 mmol, 73%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 11.93 (1H, br. s.), 10.55 (1H, s), 8.38 (1H, d, *J* = 8.5 Hz), 7.53 (1H, app. t, *J* = 8.5 Hz), 6.65 (1H, d, *J* = 8.2 Hz), 3.94 (3H, s), 1.37 (9H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 193.3, 178.7, 163.2, 142.8, 137.9, 112.1, 111.2, 104.8, 55.9, 40.5, 27.6. The above data is in agreement with that previously reported in the literature. [83]



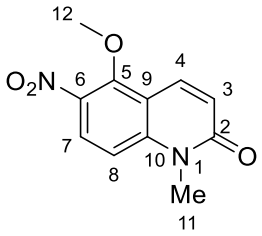
***N*-(4-bromo-2-formyl-3-methoxyphenyl)pivalamide (78)** To a solution of **77** (500 mg, 2.13 mmol, 1 eq.) in DCM (10 mL) was added NBS (378 mg, 2.13 mmol, 1 eq.) in portion. The resulting solution was stirred at RT overnight, diluted with H_2O and extracted with EtOAc (3 × 50 mL). The organic fractions were pooled, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (EtOAc:hexane ; 1:19-1:9) provided the title compound (147 mg, 0.47 mmol, 22%) as a brown solid: **mp** 77 – 78 °C (ethyl acetate-hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 11.68 (1H, br. s., *NH*), 10.41 (1H, d, *J*=0.6 Hz, *C*$_{13}$*H*), 8.53 (1H, d, *J*=9.1 Hz, *C*$_5$*H*), 7.75 (1H, d, *J*=9.5 Hz, *C*$_6$*H*), 3.99 (3H, s, *C*$_7$*H*), 1.36 (9H, s, *C*$_{12}$*H*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 193.5, 178.6, 161.4, 142.0, 141.2, 117.3, 116.9, 109.4, 63.5, 40.6, 27.5.



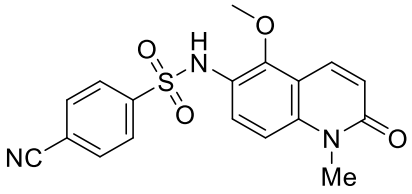
5-Methoxyquinolin-2(1*H*)-one (80) Prepared by general procedure M from **77** (1.0 g, 4.26 mmol, 1.0 eq.) to yield the title compound (672 mg, 3.84 mmol, 90% over 2 steps) as a fluffy colourless solid: **mp** 183 – 185 °C (1,4 dioxane-HCl (3M)) **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 11.71 (1H, br. s), 8.03 (1H, d, *J* = 9.6 Hz), 7.43 (1H, t, *J* = 8.2 Hz), 6.90 (1H, d, *J* = 8.3 Hz), 6.74 (1H, d, *J* = 8.3 Hz), 6.42 (1H, d, *J* = 9.9 Hz), 3.90 (3H, s); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 161.9, 155.5, 140.1, 134.1, 131.3, 120.4, 109.2, 107.7, 102.6, 55.8. The above data is in agreement with that previously reported in the literature. [119]



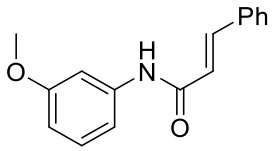
5-Methoxy-1-methylquinolin-2(1*H*)-one (81) Prepared by general procedure B from **80** (500 mg, 2.86 mmol). Purified by column chromatography (EtOAc:hexane ; 2:8) to give the title compound (345 mg, 1.83 mmol, 64 %) as a colorless solid: **mp** 132 -133 °C; **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 8.07 (1H, d, *J* = 9.6 Hz) 7.57 (1H, t, *J* = 8.5 Hz) 7.11 (1H, d, *J* = 8.6 Hz) 6.87 (1H, d, *J* = 8.1 Hz) 6.55 (1H, d, *J* = 9.9 Hz) 3.93 (3H, s) 3.60 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.5, 156.5, 141.3, 133.2, 131.1, 120.0, 111.3, 106.8, 102.8, 55.8, 29.8. The above data is in agreement with that previously reported in the literature. [87]



5-Methoxy-1-methyl-6-nitroquinolin-2(1*H*)-one (82) To a solution of **81** (270 mg, 1.43 mmol, 1 eq.) in concentrated sulfuric acid (5 mL) cooled to −5 °C was added potassium nitrate (144 mg, 1.43 mmol, 1 eq.) portionwise and the resulting yellow solution was stirred at this temperature for 1 hour before being allowed to warm to room temperature. The solution was poured over crushed ice, stirred for 10 minutes and the precipitate collected by filtration and dried under vacuum. The crude solid was purified by column chromatography (1:9–2:8 ; acetone:hexane) to give the title compound as a pale yellow solid (48 mg, 0.2 mmol, 14%): **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.23 (1H, d, *J* = 9.6 Hz, C_7H) 8.15 (1H, d, *J* = 9.9 Hz, C_4H) 7.49 (1H, d, *J* = 9.3 Hz, C_8H) 6.79 (1H, d, *J* = 9.9 Hz, $C_{13}H$) 3.99 (3H, s, $C_{12}H$) 3.66 (3H, s, $C_{13}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.8, 151.3, 144.3, 139.6, 136.2, 133.2, 122.5, 115.0, 111.3, 64.1, 29.8.



4-Cyano-*N*-(5-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (84) The required amine was synthesised by general procedure D from **82** (40 mg, 0.2 mmol) and then coupled crude *via* general procedure E. Purified by column chromatography (acetone:hexane ; 1:9 – 6:4) to give the title compound (16 mg, 0.04 mmol, 22% over 2 steps) as a pale yellow solid ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.19 (1H, s) 8.10–8.02 (2 H, m) 7.97–7.85 (3H, m) 7.39 (1H, d, *J* = 9.1 Hz) 7.26 (1H, d, *J* = 9.3 Hz) 6.61 (1H, d, *J* = 9.6 Hz) 3.59–3.56 (6H, m).



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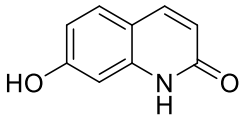
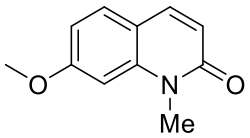
***N*-(3-methoxyphenyl)cinnamamide (85)** :A solution of 3-methoxyaniline (14.3 g, 13.06 mL, 116.2 mmol, 1 eq.) and potassium carbonate (24.1 g, 174.3 mmol, 1.5 eq.) in acetone (120 mL) and water (240 mL) was cooled to 0 °C and cinnamoyl chloride (24.2 g, 145.3 mmol, 1.25 eq.) was added portionwise over 10 minutes. Sitrring was continued for 1 hour at 0 °C and then warmed to RT and stirred for 1 hour. The resulting precipitate was filtered, washed with water and dried under vacuum to give the title compound (30.2 g, 116.4 mmol, quant.) as a brown solid: **mp** 110 – 112 °C (ethanol); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.20 (1H, s), 7.67 - 7.54 (3H, m), 7.50 - 7.38 (4H,

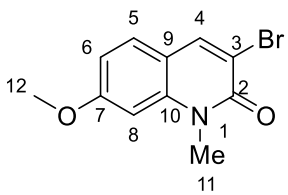
m), 7.27 - 7.20 (2H, m), 6.84 (1H, d, *J*=15.7 Hz), 6.69 - 6.63 (1H, m), 3.76 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 163.5, 159.1, 140.3, 140.1, 134.7, 129.8, 129.6, 129.0, 127.7, 122.2, 111.5, 108.7, 105.0, 54.9. The above data is in agreement with that previously reported in the literature. [120]



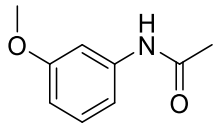
7-Hydroxyquinolin-2(1*H*)-one (86): A solution of **85** (25.3 g, 100 mmol, 1 eq.) in chlorobenzene (750 mL) was cooled to 0 °C and aluminium chloride (80 g, 600 mmol, 6 eq,) was added portionwise over 30 minutes. The resulting suspension was slowly heated to 120 °C and maintained at that temperature for 3 hours. After this time the solution was cooled to RT and poured over ice water (~ 2kg). The precipitate was removed by filtration and dried to give the title compound (14.9g, 6:1 7OH:5OH by ^{1}H NMR) which was recrystallised from ethanol to give the title compound (13:1 9.31 g, 58 mmol, 58% *contained 7% 5-OH regioisomer*) as a brown solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 11.48 (1H, br. s.), 10.08 (1H, s), 7.73 (1H, d, *J* = 9.3 Hz), 7.44 (1H, d, *J* = 8.6 Hz), 6.69 (1H, d, *J* = 2.3 Hz), 6.62 (1H, dd, *J* = 8.3, 2.3 Hz), 6.22 (1H, d, *J* = 9.6 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 162.3, 159.6, 140.8, 140.1, 129.3, 117.5, 112.4, 111.5, 99.8. The above data is in agreement with that previously reported in the literature. [120]



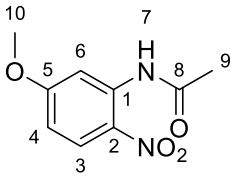
7-Methoxy-1-methylquinolin-2(1*H*)-one (87) : To a suspension of **86** (7.3 g, 45 mmol, 1 eq.) in dry DMF (100 mL) under an argon atmosphere was added NaH (60%, 4.35 g , 108.8 mmol, 2.4 eq.) in 4 portions over 20 minutes. Upon completion of gas evolution iodomethane (15.45 g, 6.8 mL, 108.8 mmol, 2.4 eq.) was added dropwise portion and the resulting solution was stirred overnight. Excess sodium hydride was quenched by the addition of water (20 mL) and the solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate (200 mL), washed with water and then brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and then concentrated under reduced pressure. The crude solid was purified by column chromatography 7:3 hexane:acetone) to give the title compound (4.63g, 24 mmol, 54%) as a pale pink solid ; **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.61 (1H, d, *J* = 9.6 Hz), 7.48 (1H, d, *J* = 8.3 Hz), 6.86 - 6.82 (1H, m), 6.81 (1H, d, *J* = 2.3 Hz), 6.57 (1H, d, *J* = 9.3 Hz), 3.94 (3H, s), 3.71 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$) δ ppm 162.8, 161.8, 141.7, 138.7, 130.0, 118.6, 114.9, 109.6, 98.7, 55.6, 29.6. The above data is in agreement with that previously reported in the literature. [121]



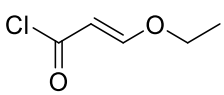
3-Bromo-7-methoxy-1-methylquinolin-2(1*H*)-one (88) : **87** (2g, 10.10 mmol, 1 eq.) was dissolved in DMF (25 mL) and NBS (1.98 g, 11.11 mmol, 1.1 eq.) was added in one portion. The resulting solution was stirred overnight and then poured onto crushed ice and stirred for 30 minutes. The precipitate was filtered, washed with water (~100 mL) and dried under vacuum to the title compound (2.50 g, 9.31 mmol, 92%) as a pink solid: **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 8.06 (1H, s, *C*$_4$*H*), 7.45 (1H, d, *J*=8.6 Hz, *C*$_5$*H*), 6.87 (1H, dd, *J*=8.6, 2.3 Hz, *C*$_6$*H*), 6.79 (1H, d, *J*=2.3 Hz, *C*$_8$*H*), 3.95 (3H, s, *C*$_{12}$*H*), 3.78 (3H, s, *C*$_{11}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$) δ ppm 161.7, 157.6, 140.8, 140.6, 129.7, 114.1, 112.3, 110.8, 98.8, 55.7, 30.8.



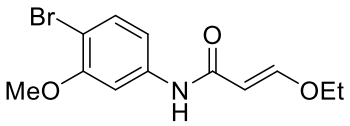
***N*-(3-methoxyphenyl)acetamide (94) :** 3-methoxyaniline (10g, 50.4 mmol, 1 eq.) was dissolved in acetic acid (20 mL) and acetic anhydride (5.7 g, 55.8 mmol, 1.1 eq.) was added dropwise. The resulting solution was stirred overnight and then poured onto crushed ice and stirred for 30 mins. The resulting precipitate was filtered, washed with water and dried under vacuum to give the title compound (13.2 g, 80.9 mmol, quant.) as a brown solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.50 (1H, br. s.), 7.28 (1H, s), 7.21 (1H, t, *J* = 8.1 Hz), 6.99 (1H, d, *J* = 7.8 Hz), 6.67 (1H, dd, *J* = 8.2, 1.6 Hz), 3.80 (3H, s), 2.18 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm , 161.7, 141.7, 138.7, 130.1, 118.6, 109.7, 98.8, 55.7, 29.5. The above data is in agreement with that previously reported in the literature. [86]



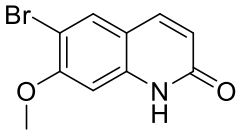
***N*-(5-methoxy-2-nitrophenyl)acetamide (95a) :** To a solution of **94** (5 g, 30.3 mmol, 1 eq.) in acetic acid (90 mL) at 0 °C was added a premixed solution of nitric acid (70%, 5.45 g, 3.9 mL, 60.6 mmol, 2 eq.) in acetic anhydride (6 mL) dropwise maintaining the temperature below 5 °C. Stirring was continued for 3 hours at 0 °C and the solution was poured onto crushed ice. The aqueous layer was extracted with ethyl acetate (3 × 100 mL) and the combined organic fractions were pooled, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography (EtOAc:hexane ; 1:9-1:1) to give the title compound (2.58 g, 12.2 mmol, 41 %) as bright orange crystals: **mp** 122 – 124 °C (ethyl acetate-hexane) **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 10.78 (1H, br. s., *NH*), 8.44 (1H, d, *J* = 2.8 Hz, *C*$_6$*H*), 8.21 (1H, d, *J* = 9.3 Hz, *C*$_3$*H*), 6.66 (1H, dd, *J* = 9.3, 2.8 Hz, *C*$_4$*H*), 3.92 (3H, s, *C*$_{10}$*H*), 2.30 (3H, s, *C*$_9$*H*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 169.4, 165.8, 137.8, 129.4, 128.1, 110.6, 104.1, 56.1, 25.9. The above data is in agreement with that previously reported in the literature. [85]



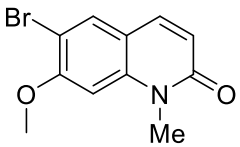
(*E*)-3-ethoxyacryloyl chloride (97) Oxalyl chloride (12.9 mL, 150 mmol, 1.5 eq.) was cooled to 0 °C and ethyl vinyl ether (9.56 mL, 100 mmol, 1 eq.) was added dropwise. The solution was allowed to warm to RT and then stirred overnight. The volatiles were removed under reduced pressure and the residue was heated under a positive pressure of argon to 110 °C to give the title compound (9.73 g, 73 mmol, 73%) as a black oil that was used without further purification.



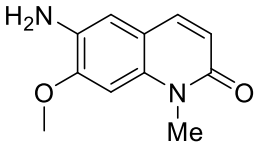
(*E*)-*N*-(4-bromo-3-methoxyphenyl)-3-ethoxyacrylamide (98) To a solution of 3-methoxy-4-bromoaniline (1.01 g, 5.00 mmol, 1 eq.) and pyridine (474 mg, 484 µL, 6 mmol, 1.2 eq.) in DCM (20 mL) was added **97** (432 mg, 6 mmol, 1.2 eq.). The solution was stirred for 3 hours, the solvent removed under reduced pressure and the residue purified by column chromatography (1:9–6:4 ; ethyl acetate:Hexane) to give the title compound (885 mg, 2.95 mmol, 59%) as sticky brown solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 9.86 (1H, s), 7.55–7.47 (2H, m), 7.44 (1H, d, *J* = 8.6 Hz), 7.11 (1H, dd, *J* = 8.6, 2.3 Hz), 5.51 (1H, d, *J* = 12.1 Hz), 3.96 (2H, q, *J* = 6.9 Hz), 3.83 (3H, s), 1.27 (3 H, q, *J* = 7.6 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 165.2, 161.7, 156.2, 138.8, 132.9, 112.6, 105.6, 104.5, 98.7, 67.6, 56.2, 14.7.



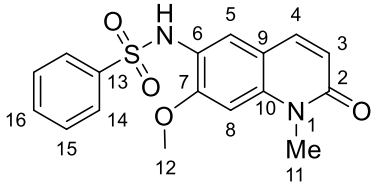
6-Bromo-7-methoxyquinolin-2(1*H*)-one (99) Concentrated sulphuric acid (8 mL) was cooled to 0 °C and **98** (880 mg, 2.93 mmol) was added portionwise. The dark solution was allowed to stir for 20 minutes and was then poured onto ice. The resulting precipitate was filtered, washed with water and dried under vacuum to afford the title compound (745 mg, 2.93 mmol, quant.) as a brown solid: **mp** >300 °C (water); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.94 (1H, s), 7.80 (1H, d, *J* = 9.6 Hz), 6.94 (1H, s), 6.37 (1H, d, *J* = 9.6 Hz), 3.89 (3H, s).



6-Bromo-7-methoxy-1-methylquinolin-2(1*H*)-one (100) Prepared by general procedure B from **99** (200 mg, 0.81 mmol, 1 eq.) Purification by column chromatography (1:4 ethyl acetate:hexane) gave the title compound (132 mg, 0.50 mmol, 56%) as a pale yellow solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.00 (1H, s), 7.81 (1H, d, *J* = 9.6 Hz), 7.04 (1H, s), 6.49 (1H, d, *J* = 9.6 Hz), 4.02 (3H, s), 3.64 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.3, 157.6, 141.0, 137.7, 132.3, 119.5, 115.6, 105.9, 97.1, 56.5, 29.6.

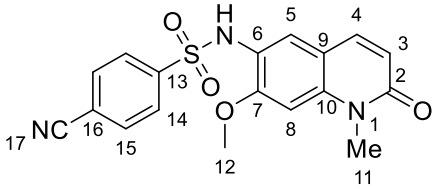


6-amino-7-methoxy-1-methylquinolin-2(1*H*)-one (93): Prepared by general procedure J from **100** (200 mg, 0.76 mmol, 1.0 eq.). The resulting residue was purified by column chromatography (7:3–3:7 hexane:acetone) to give the title compound as a solution in NMP: Relevant **^{1}H NMR** (400 MHz, DMSO-*d_6*) δ ppm 7.63 (1H, d, *J* = 9.3 Hz) 6.86 (2H, d, *J* = 11.4 Hz) 6.36 (1H, d, *J* = 9.3 Hz) 4.77 (2H, s) 3.95 (3H, s) 3.60 (3H, s).



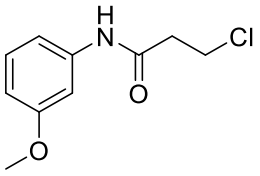
***N*-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (101)**

Prepared by general procedure E from a solution of **93** in NMP (39 mg, 0.19 mmol). Purified by column chromatography (acetone:hexane ; 3:7 – 1:1) to give the title compound (24 mg, 0.07 mmol, 37% over 2 steps) as a colourless solid: **mp** 236-237 °C (acetone-hexane); **IR** (neat) ν_{max}: 3200, 1697, 1591, 1430, 1397, 1350, 1324, 1250, 1165, 1119, 1054, 823, 777, 666, 634, 598, 558, 506 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-*d_6*): δ ppm 9.62 (1H, s, *NH*), 7.83 (1H, d, *J* = 9.4 Hz, *C_4H*), 7.71 (2H, d, *J* = 7.3 Hz), 7.64–7.48 (4H, m, *$C_{14}H/C_{15}H$*), 6.83 (1H, s, *C_8H*), 6.45 (1H, d, *J* = 9.3 Hz, *C_3H*), 3.58 (6H, app. s, *$C_{12}H/ C_{11}H$*); **^{13}C NMR** (126 MHz, DMSO-*d_6*): δ ppm 161.2, 155.2, 140.4, 139.5, 138.7, 132.5, 128.8, 126.6, 126.3, 120.3, 118.2, 113.3, 97.5, 55.8, 29.2; **HRMS**: *m/z* ESI- [Found (M-H)$^-$ 343.0739, $C_{17}H_{16}N_2O_4S$ requires (M-H)$^-$ 343.0753]; **HPLC**: Retention time (system A): t_R= 7.79 min. Purity: >95%.

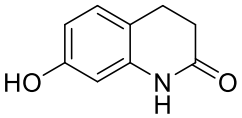


4-Cyano-*N*-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (102)

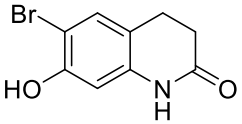
Prepared by general procedure E from a solution of **93** in NMP (39 mg, 0.19 mmol). Purified by column chromatography (acetone:hexane ; 3:7 – 6:4) to give the title compound (15 mg, 0.04 mmol, 21% over 2 steps) as a colourless solid: **mp** 291-292 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-*d_6*): δ ppm 9.99 (1H, br. s, *NH*), 8.03 (2H, d, *J* = 8.3 Hz, *$C_{15}H$*), 7.89–7.80 (3H, m, *$C_{14}H/C_4H$*), 7.60 (1H, s, *C_5H*), 6.83 (1H, s, *C_8H*), 6.46 (1H, d, *J* = 9.6 Hz, *C_3H*), 3.58 (3H, s, *$C_{12}H$*), 3.54 (3H, s, *$C_{11}H$*); **^{13}C NMR** (126 MHz, DMSO-*d_6*): δ ppm 161.2, 155.6, 144.7, 140.0, 138.7, 133.0, 127.6, 127.4, 119.3, 118.3, 117.8, 114.8, 113.4, 97.6, 55.7, 29.2; **HRMS**: *m/z* NSI+ [Found (M+H)$^+$ 370.0855, $C_{17}H_{16}N_2O_4S$ requires (M+H)$^+$ 370.0854]; **HPLC**: Retention time (system A): t_R= 6.17 min. Purity: >95%.



3-Chloro-*N*-(3-methoxyphenyl)propanamide (103) – 3-methoxyaniline (10.00 g, 81.3 mmol, 1 eq.) was dissolved in toluene (20 mL) and $NaHCO_3$ (10.29 g, 122 mmol, 1.5 eq.) was added. The resulting suspension was cooled to 0 °C and 3-chloropropionyl chloride (10.33 g, 81.3 mmol, 1 eq.) was added dropwise with rapid stirring. On completion a further 10 mL of toluene was added and the suspension was heated to 50 °C for 2 hours and then poured with stirring onto 3N HCl (50 mL). The precipitate was filtered, washed with H_2O and toluene and then dried to give the title compound (13.70 g, 64.2mmol, 79%) as a brown solid that was used without further purification. Analytical sample purified by column chromatography (EtOAc:hexane, 1:4). 200 mg of crude solid gave 187 mg of the title compound as white needles: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.33 (1H, br. s.), 7.23 (1H, t, *J* = 2.5 Hz), 7.14 (1H, t, *J* = 8.2 Hz), 6.91 (1H, d, *J* = 7.6 Hz), 6.61 (1H, dd, *J* = 8.5, 1.9 Hz), 3.81 (2H, t, *J* = 6.4 Hz), 3.73 (3H, s), 2.74 (2H, t, *J*=6.4 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 167.7, 160.2, 139.0, 130.4, 112.1, 110.6, 106.0, 55.3, 40.6, 39.9. The above data is in agreement with that previously reported in the literature. [122]

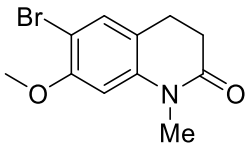


7-Hydroxy-3,4-dihydroquinolin-2(*1H*)-one (104) - **103** (15.0 g, 70.2 mmol. 1.0 eq.) was dissolved in *N,N*-dimethylacetamide (12 mL) and cooled to 0 °C. $AlCl_3$ (37.4 g, 281 mmol, 4 eq.) was added portionwise and the resulting solution was fitted with a calcium chloride guard tube and heated to 150 °C for 2 hours. On completion the reaction was allowed to cool to ~60 °C and then poured over ice (~300 g) and stirred for 20 mins. The precipitate was filtered, washed with H_2O dried and then crystallized (EtOH) to give the title compound (6.16 g, 37.80 mmol, 54%) as a pale pink solid**: ^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.93 (1H, br. s), 9.25 (1H, s), 6.92 (1H, d, *J* = 7.9 Hz), 6.33 (1H, d, *J* = 2.5 Hz), 6.31 (1H, dd, *J* = 8.2, 2.5 Hz), 2.73 (2H, t, *J* = 7.4 Hz), 2.39 (2H, t, *J* = 7.3 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 170.3, 156.4, 139.0, 128.1, 113.7, 108.6, 102.3, 30.8, 23.9. The above data is in agreement with that previously reported in the literature. [89]

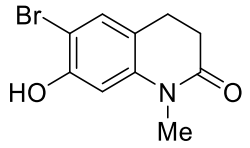


6-Bromo-7-hydroxy-3,4-dihydroquinolin-2(*1H*)-one (105) – Synthesised according to the method of Hu *et al* from **104** (2.0 g, 12.3 mmol) and NBS (2.4 g, 13.5 mmol, 1.1 eq.) to give the title compound (1.71 g, 7.12 mmol, 58%) as a colourless powder: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.08 (2H,app. s), 7.25 (1H, s), 6.54 (1H, s), 2.76 (2H, t, *J* = 7.6 Hz), 2.39 (2H, t, *J*= 7.6 Hz) ; **^{13}C NMR**

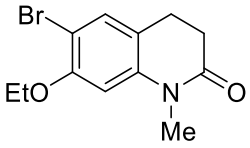
(126 MHz, DMSO-d_6): δ ppm 170.2, 152.8, 138.5, 131.2, 115.9, 103.1, 101.0, 30.5, 23.6. The above data is in agreement with that previously reported in the literature. [90]



6-Bromo-7-methoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (106) – 105 (1.1 g, 4.6 mmol, 1 eq.) was dissolved in dry DMF (9 mL) under argon and NaH (60% in mineral oil, 405 mg, 10.1 mmol, 2.2 eq.) was added in 1 portion with rapid stirring. After gas evolution had ceased the solution was cooled to 0 °C and iodomethane (1.5 mL, large excess) was added dropwise. The resulting solution was stirred overnight and then poured onto ice and stirred for 20 mins. The precipitate was filtered, washed with water and crystallized (MeOH) to give the title compound (905 mg, 3.36 mmol, 73%) as colourless needles: **mp** 159 – 161 °C (methanol); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.34 (1H, s), 6.55 (1H, s), 3.94 (3H, s), 3.39 (3H, s), 2.85 (2H, t, *J* = 7.9 Hz), 2.66 (2H, t, *J* = 7.9 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 170.1, 155.2, 141.0, 131.8, 119.6, 104.4, 100.0, 56.5, 31.7, 29.7, 24.2. The above data is in agreement with that previously reported in the literature. [90]

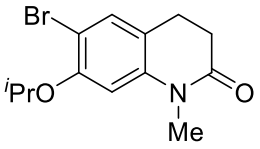


6-Bromo-7-hydroxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (107) – 106 (500 mg, 1.85 mmol, 1 eq.) was dissolved in dry THF (9 mL) and the resulting solution was cooled to – 78 °C. BBr_3 (1.0 M in heptane, 5.56 mmol, 3 eq.) was added dropwise and the solution was then allowed to warm to RT and stirred for 3 hours. The solution was poured over ice and the precipitate filtered, washed with H_2O and dried to give the title compound (287 mg, 1.12 mmol, 61%) as yellow solid: **mp** 191 – 193 °C (water); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.16 (1H, s), 7.31 (1H, s), 6.66 (1H, s), 3.18 (3H, s), 2.76 (2H, t, *J* = 7.4 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 169.3, 152.9, 140.6, 130.9, 118.3, 103.5, 101.5, 31.3, 28.9, 23.2.

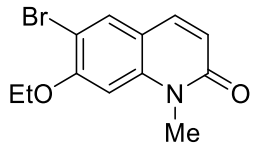


6-Bromo-7-ethoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (108a) – 107 (100 mg, 0.39 mmol, 1 eq.) was dissolved in dry DMF (2 mL) under an argon atmosphere and NaH (60% in mineral oil, 17 mg, 0.43 mmol, 1.1 eq.) was added in one portion. Upon completion of gas evolution iodomethane (61 mg, 0.43 mmol, 1.1 eq.) was added in one portion and the resulting solution was stirred overnight. H_2O (10 mL) was added and the resulting solution was extracted with EtOAc (3 × 20 mL). The organic fractions were pooled, washed with saturated brine (3 × 20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give the title compound

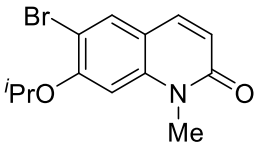
(112 mg, 0.39 mmol, quant.) as a pale yellow solid: **mp** 109 -111 °C (DMF-water); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.31 (1H, s), 6.53 (1H, s), 4.12 (2H, q, *J* = 6.9 Hz), 2.83 (2H, t, *J* = 7.9 Hz), 2.63 (2H, t, *J* = 7.9 Hz), 1.49 (3H, t, *J* = 6.9 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 170.3, 154.7, 140.9, 131.7, 119.7, 105.1, 101.6, 65.5, 31.8, 29.6, 24.2, 14.8.



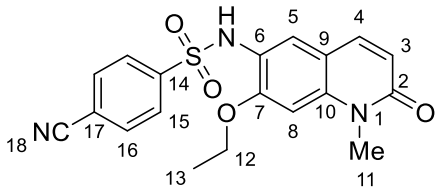
6-Bromo-7-isopropoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (108b) Prepared as per compound **108a** from **107** (100 mg, 0.39 mmol) and 2-iodopropane (74 mg, 0.43 mmol, 1.1 eq.) to give the title compound (73 mg, 0.25 mmol, 63 %): **mp** 101 -103 °C (DMF-water); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.23 (1H, s), 6.49 (1H, s), 4.45 (1H, spt, *J* = 6.0 Hz), 3.25 (3H, s), 2.75 (2H, t, *J* = 7.9 Hz), 2.56 (2H, t, *J* = 7.9 Hz), 1.32 (6H, d, *J* = 6.0 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 170.2 , 153.9, 140.8, 131.8, 120.4, 107.0, 104.6, 73.3, 31.8, 29.7, 24.3, 22.1.



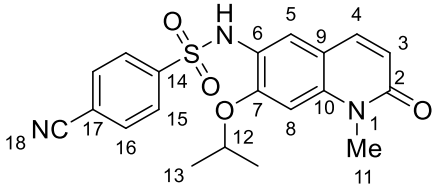
6-Bromo-7-ethoxy-1-methylquinolin-2(1*H*)-one (109a) Prepared by general procedure I from **108a** (80 mg, 0.28 mmol) to give the title compound (79 mg, 0.28 mmol, quant) as a pale yellow solid: **mp** 174 - 175 °C (DCM); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.06 (1H, s), 7.45 (1H, d, *J* = 8.8 Hz), 6.86 (1H, dd, *J* = 8.5, 2.2 Hz), 6.80 (1H, d, *J* = 2.2 Hz), 4.17 (2H, q, *J* = 6.9 Hz), 3.78 (3H, s), 1.51 (3H, t, *J* = 6.9 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.4, 158.8, 141.1, 140.3, 129.5, 114.7, 113.7, 110.7, 99.4, 64.0, 31.1, 14.3.



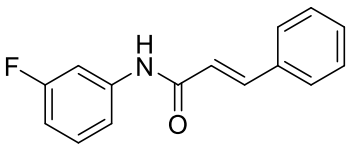
6-Bromo-7-isopropoxy-1-methylquinolin-2(1*H*)-one (109b) Prepared by general procedure I from **109b** (60 mg, 0.20 mmol) to give the title compound (60 mg, 0.20 mmol, quant) as a pale yellow solid: **mp** 1157 - 159 °C (DCM); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.02 (1H, s), 7.51 (1H, d, *J* = 8.9 Hz), 6.84 (1H, dd, *J* = 8.6, 2.1 Hz), 6.80 (1H, d, *J* = 2.1 Hz), 4.45 (1H, spt, *J* = 6.0 Hz), 1.32 (6H, d, *J* = 6.0 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.0, 158.8, 139.6, 139.5, 130.0, 114.9, 114.6, 110.9, 99.0, 65.2, 29.8, 22.2.



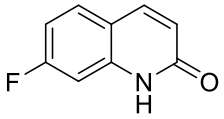
4-Cyano-*N*-(7-ethoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (111) The required amine was prepared by general procedure J from **109a** (50 mg, 0.18 mmol). Purification of the residue by column chromatography (acetone:hexane ; 3:7-4:6) provided the amine as a solution in NMP. Coupling of the crude amine by general procedure E and purification of the residue by column chromatography (acetone:hexane ; 4:6 – 6:4) provided the title compound (12 mg, 0.03 mmol, 17% over 2 steps): **mp** 228 – 230 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.96 (1H, br. s, *NH*), 8.03 (2H, d, *J* = 7.9 Hz, $C_{16}H$), 7.87 (1H, d, *J* = 9.5 Hz, C_4H), 7.81 (2H, d, *J* = 7.9 Hz, $C_{15}H$), 7.63 (1H, s, C_5H), 6.81 (1H, s, C_8H), 6.45 (1H, d, *J* = 9.1 Hz, C_3H), 3.87 (2H, q, *J* = 6.3 Hz, $C_{12}H$), 3.56 (3H, s, $C_{11}H$), 1.06 (3H, t, *J* = 6.8 Hz, $C_{13}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.2, 154.8, 144.7, 140.0, 138.7, 133.0, 127.7, 127.4, 119.3, 118.2, 117.7, 114.8, 113.3, 97.9, 64.0, 29.2, 13.8; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 382.0862, $C_{19}H_{17}N_3O_4S$ requires $(M-H)^-$ 382.0862]; **HPLC**: Retention time (system A): t_R= 8.08 min. Purity: >95%.



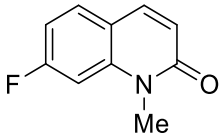
4-Cyano-*N*-(7-isopropoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (112) The required amine was prepared by general procedure J from **109b** (50 mg, 0.17 mmol). Purification of the residue by column chromatography (acetone:hexane ; 3:7-4:6) provided the amine as a solution in NMP. Coupling of the crude amine by general procedure E and purification of the residue by column chromatography (acetone:hexane ; 4:6 – 6:4) provided the title compound (16 mg, 0.04 mmol, 24% over 2 steps): **mp** 209-210 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 9.87 (1H, br. s. *NH*), 8.03 (2H, d, *J* = 8.6 Hz, $C_{16}H$), 7.84 - 7.78 (3H, m, $C_{15}H/C_4H$), 7.64 (1H, s, C_5H), 6.80 (1H, s, C_8H), 6.44 (1H, d, *J* = 9.6 Hz, C_3H), 4.67 (1H, spt, *J* = 6.0 Hz, $C_{12}H$), 3.55 (3H, s, $C_{11}H$), 1.01 (6H, d, *J* = 6.1 Hz, $C_{13}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.2, 153.5, 144.8, 139.9, 138.7, 133.1, 127.7, 127.5, 119.8, 118.1, 117.7, 114.8, 113.1, 98.5, 70.0, 29.2, 20.9; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 396.1015, $C_{20}H_{19}N_3O_4S$ requires $(M-H)^-$ 396.1018]; **HPLC**: Retention time (system A): t_R= 9.51 min. Purity: >95%.



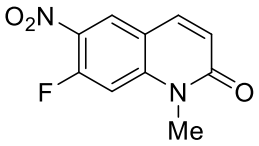
***N*-(3-fluorophenyl)cinnamamide (114)** Prepared by general procedure K from 2-fluoroaniline (4.0 g, 36.03 mmol) to give the title compound (8.07 g, 33.51 mmol, 93%) as a colourless solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.43 (1H, s), 7.82 - 7.58 (4H, m), 7.50 - 7.32 (5H, m), 6.95 - 6.87 (1H, m), 6.83 (1H, d, *J* = 15.7 Hz). The above data is in agreement with that previously reported in the literature. [123]



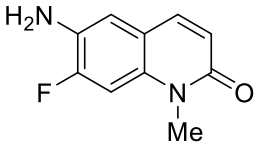
7-Fluoroquinolin-2(1*H*)-one (115) Prepared by general method L from **114** (5.00 g, 20.58 mmol). Crystallised (EtOH) to give the title compound (2.05 g, 12.6 mmol, 61%) as a pink solid (contains 24% 5-F isomer by ^{1}H NMR): **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 11.82 (1 H, br. s.), 7.91 (1 H, d, *J* = 9.6 Hz), 7.73 (1 H, dd, *J* = 8.5, 6.2 Hz), 7.10 – 6.99 (2H, m), 6.46 (1H, d, *J* = 9.6 Hz). Used without further purification.



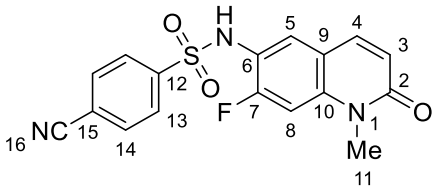
7-Fluoro-1-methylquinolin-2(1*H*)-one (116) Prepared by general procedure B from **115** (1.00 g, 6.13 mmol). Purified by column chromatography (acetone:hexane ; 1:19 – 1:9) to give the title compound (836 mg, 4.72 mmol, 77%) (contains 11% 5-F isomer by ^{1}H NMR) as a pale pink solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.91 (1H, d, *J* = 9.3 Hz), 7.79 (1H, dd, *J* = 8.6, 6.6 Hz), 7.40 (1H, dd, *J* = 11.6, 2.3 Hz), 7.14 (1H, td, *J* = 8.6, 2.5 Hz), 6.57 (1H, d, *J* = 9.6 Hz), 3.58 (3H, s). Used without further purification.



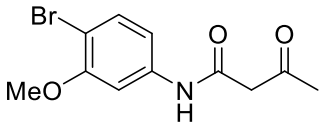
7-Fluoro-1-methyl-6-nitroquinolin-2(1*H*)-one (117) Prepared by general procedure C from **116** (700 mg, 3.95 mmol). Crystallised (EtOH) to give the title compound (300 mg, 1.34 mmol, 34%) as a yellow solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.51 (1H, d, *J* = 8.3 Hz), 7.88 (1H, d, *J* = 9.6 Hz), 7.51 (1H, d, *J* = 14.1 Hz), 6.53 (1H, d, *J* = 9.6 Hz), 3.40 (3H, s).



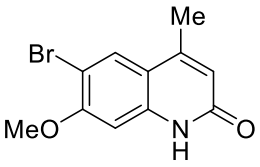
7-Fluoro-1-methyl-6-aminoquinolin-2(1*H*)-one (118) Prepared by general procedure D from **117** (100 mg, 0.45 mmol) to give the title compound (33 mg, 0.17 mmol, 39%) as a bright yellow solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.52 (1H, d, *J* = 9.8 Hz), 7.06 (1H, d, *J* = 12.6 Hz), 6.95 (1H, d, *J* = 9.1 Hz), 6.64 (1H, d, *J* = 9.5 Hz), 3.78 (2H, br. s.), 3.66 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.9, 153.8 (d, *J* = 244.7 Hz), 137.7, 133.7 (d, *J* = 10.1 Hz), 130.2 (d, *J* = 13.7 Hz), 121.1 (d, *J* = 3.7 Hz), 117.7 (d, *J* = 2.7 Hz), 114.8 (d, *J* = 5.5 Hz), 101.7 (d, *J* = 24.7 Hz), 29.6.



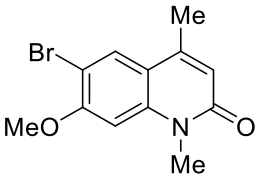
4-Cyano-*N*-(7-fluoro-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (119) Prepared according to general procedure E from **118** (20 mg, 0.10 mmol). Purified by column chromatography (acetone:hexane ; 3:7 – 6:4) to give the title compound (31 mg, 0.08 mmol, 83%) as a colourless solid: **mp** >300 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.52 (1H, br. s, *NH*), 8.06 (2H, d, *J* = 8.6 Hz, $C_{14}H$), 7.92 (1H, d, *J* = 9.6 Hz, C_4H), 7.87 (2H, d, *J* = 8.6 Hz, $C_{13}H$), 7.65 (1H, d, *J* = 8.6 Hz, C_5H), 7.41 (1H, d, *J* = 12.6 Hz, C_8H), 6.59 (1H, d, *J* = 9.6 Hz, C_3H), 3.53 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.9, 157.8 (d, *J* = 250.2 Hz), 143.9, 139.7 (d, *J* = 11.0 Hz), 138.5, 133.4, 128.3, 127.4, 120.8, 117.6 , 116.9, 115.3, 102.8, 102.6, 29.5; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 356.508 $C_{17}H_{12}N_3O_3SF$ requires $(M-H)^-$ 356.505]; **HPLC**: Retention time (system A): t_R= 7.79 min. Purity: >95%.



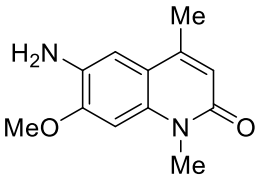
***N*-(4-bromo-3-methoxyphenyl)-3-oxobutanamide (120)** To a solution of 4-bromo-3-methoxyaniline (2.5 g, 12.32 mmol, 1 eq.) in xylenes (25 mL) at 110 °C was added 2,2,6-Trimethyl-4*H*-1,3-dioxin-4-one (1.93 g, 13.55 mmol, 1.1 eq.). The solution was stirred for 2 hours and allowed to cool to RT. The solvents were removed under reduced pressure and the residue purified by column chromatography (8:2–6:4; hexane:ethyl acetate) to yield the title compound (2.32 g, 8.14 mmol, 66%) as a brown oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 9.07 (1H, br. s), 7.30 (1H, t, *J* = 2.2 Hz), 7.24 (1H, t, *J* = 8.1 Hz), 7.06 (1H, dd, *J* = 8.0, 1.1 Hz), 6.70 (1H, dd, *J* = 8.2, 1.9 Hz), 3.83 (3H, s), 3.61 (2H, s), 2.35 (3H, s).



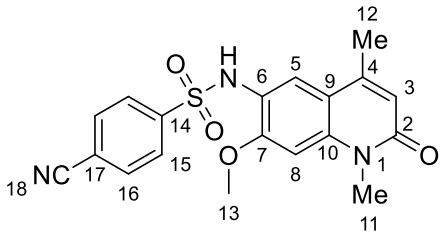
6-Bromo-7-methoxy-4-methylquinolin-2(1*H*)-one (121) A mixture of **120** (1.21 g, 4.05 mmol) and polyphosphoric acid (10 g) was heated at 90 °C for 2 hours. The reaction mixture was allowed to cool to approximately 60 °C and then ice was added until a freely stirring mixture was achieved. The precipitate was isolated by filtration and dried under vacuum to yield the title compound (1.05 g, 3.90 mmol 96%) as a colourless solid: **mp** 257 - 259 °C (water); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.87 (1H, s), 6.94 (1H, s), 6.27 (1H, s), 3.89 (3H, s), 2.38 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.7, 156.5, 147.2, 139.7, 128.7, 118.8, 114.7, 104.5, 98.1, 56.3, 18.4.



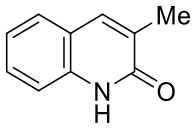
6-Bromo-7-methoxy-1,4-dimethylquinolin-2(1*H*)-one (122) was prepared according to general procedure B from **121** (500 mg, 1.88 mmol). Product precipitated by addition of H_2O, filtered and dried to give the title compound (275 mg, 0.98 mmol, 52%) as a pale yellow solid: **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 7.92 (1H, s), 7.02 (1H, s), 6.41 (1H, s), 4.02 (3H, s), 3.62 (3H, s), 2.39 (3H, d, *J* = 0.5 Hz); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 162.2, 157.3, 145.3, 140.7, 129.4, 119.1, 116.3, 105.7, 97.2, 56.4, 28.7, 18.4.



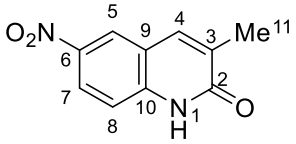
6-Amino-7-methoxy-1,4-dimethylquinolin-2(1*H*)-one (123) Prepared by general procedure J from **122** (100 mg, 0.35 mmol, 1 eq.).The resulting residue was purified by column chromatography (7:3–3:7 hexane:acetone) to give the title compound as a crude solution solution in NMP. Relevant **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 6.96 (1H, s), 6.87 (1H, s), 6.29 (1H, s), 4.82–4.76 (2H, br. s), 3.95 (3H, s), 3.59 (3H, s), 2.31 (3H, s).



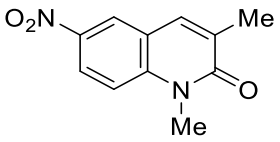
4-Cyano-*N*-(7-methoxy-1,4-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (124) Prepared by general procedure E from a solution of **123** in NMP (75 mg, 0.35 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 1:1) to give the title compound (28 mg, 0.07 mmol, 20% over 2 steps) as a pale yellow solid: **mp** 260 - 262 °C (acetone-hexane);**^{1}H NMR** (500 MHz, DMSO-*d*$_6$): δ ppm 10.05 (1H, br. s, *NH*), 8.04 (2H, d, *J* = 8.2 Hz, *C*$_{16}$*H*), 7.83 (2H, d, *J* = 8.5 Hz, *C*$_{15}$*H*), 7.52 (1H, s, *C*$_5$*H*), 6.84 (1H, s, *C*$_8$*H*), 6.40 (1H, s, *C*$_3$*H*), 3.56 (6H, m, *C*$_{11}$*H/ C*$_{13}$*H*), 2.34 (3H, s, *C*$_{12}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.9, 155.5, 145.8, 144.7, 139.7, 133.0, 127.5, 124.1, 119.3, 118.0, 117.7, 114.8, 113.8, 97.8, 55.7, 29.0, 18.3; **HRMS**: *m/z* NSI+ [Found $(M+H)^+$ 384.1012 $C_{19}H_{17}N_3O_4S$ requires M^+ 384.0940]; **HPLC**: Retention time (system A): t_R= 7.21 min. Purity: >95%.



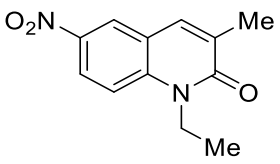
3-Methylquinolin-2(1*H*)-one (126) Prepared according to general procedure A from 3-methylquinoline (5.0 g, 35.0 mmol) to give the title compound (3.78 g, 23.8 mmol, 69%) as a colourless solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.72 (1H, br. s.), 7.75 (1H, s), 7.56 (1H, d, *J* = 7.8 Hz), 7.41 (1H, t, *J* = 8.3 Hz),7.29 (1H, d, *J* = 8.3 Hz), 7.14 (1H, t, *J* = 7.5 Hz), 2.09 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 162.4, 137.9, 136.3, 129.8, 129.0, 126.9, 121.6, 119.4, 114.7, 16.5. The above data is in agreement with that previously reported in the literature. [124]



3-Methyl-6-nitroquinolin-2(1*H*)-one (127) Prepared according to the procedure or Fish *et al* from **126** (3.0 g, 18.9 mmol) to give the title compound (3.0 g, 14.6 mmol, 77%) as a pale yellow solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ 12.12 (1H, s, *NH*), 8.63 (1H, s, C_5H), 8.38 (1H, d, *J* = 8.7 Hz, C_7H), 8.08 (s, 1H, C_3H), 7.66 (1H, d, *J* = 8.8 Hz, C_8H), 2.16 (3H, s, $C_{11}H$); **^{13}C NMR** (125 MHz, DMSO-d_6): δ 161.8, 143.0, 141.2, 135.1, 131.4, 123.9, 123.6, 119.9, 115.8, 17.0. The above data is in agreement with that previously reported in the literature. [92]

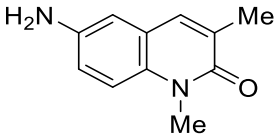


1,3-Dimethyl-6-nitroquinolin-2(1*H*)-one (128a) Prepared according to general procedure B from **127** (1.0 g, 4.90 mmol) to give the title compound (588 mg, 2.70 mmol, 54%) as a pale yellow solid: **mp** 196 -198 °C (DMF-water);;**^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.63 (1H, s), 8.35 (1H, d, *J* = 8.8 Hz), 8.03 (1H, s), 7.7 (1H, d, *J* = 8.8 Hz), 3.71 (3H. s) 2.16 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 161.8, 142.9, 141.4, 135.3, 131.2, 123.9, 123.5, 119.7, 115.7, 30.1, 17.4. The above data is in agreement with that previously reported in the literature. [92]

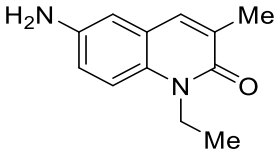


1-Ethyl-3-methyl-6-nitroquinolin-2(1*H*)-one (128b) Prepared by general procedure B from **127** (500 mg, 2.45 mmol) to give the title compound (470 mg, 2.0 mmol, 83%) as a pale yellow solid: **mp** 241 – 243 °C (DMF-water); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.45 (1H, d, *J* = 2.5 Hz), 8.37 (1H, dd, *J* = 9.5, 2.5 Hz), 7.66 (1H, s), 7.45 (1H, d, *J* = 9.5 Hz), 4.43 (2H, q, *J* = 7.1 Hz), 2.32 (3H, d, *J* = 0.9

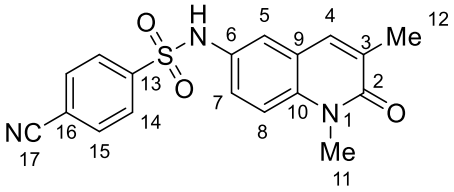
Hz), 1.41 (3H, t, *J* = 7.1 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.2, 142.1, 141.9, 135.1, 132.8, 124.0, 123.9, 120.5, 114.3, 38.3, 17.7, 12.7.



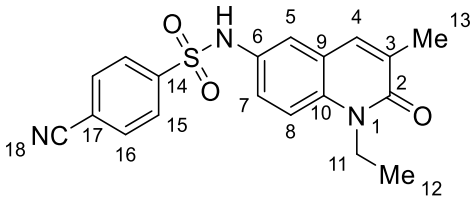
6-Amino-1,3-dimethylquinolin-2(1*H*)-one (129a) Prepared according to the procedure of Fish *et al* from **128a** (500 mg, 2.29 mmol) to give the title compound (319 mg, 1.70 mmol, 74%) as a golden powder: **^{1}H NMR** (500 MHz, DMSO-d_6): δ 7.55 (1H, s), 7.21 (1H, d, *J* = 8.8 Hz), 6.87 (1H, d, *J* = 8.8 Hz), 6.71 (1H, s), 5.04 (2H, br. s), 3.56 (3H, s) 2.08 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 160.9, 143.6, 135.0, 130.5, 128.7, 120.9, 117.7, 115.0, 110.0, 29.2, 17.5. The above data is in agreement with that previously reported in the literature. [92]



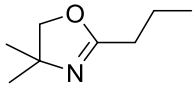
6-Amino-1-ethyl-3-methylquinolin-2(1*H*)-one (129b) Prepared by general procedure D from **128b** (250 mg, 1.08 mmol) to give the title compound (135 mg, 0.67 mmol, 62%) as a yellow solid: **mp** 173 - 175 °C (DCM); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.42 (1 H, s), 7.21 (1 H, d, *J* = 8.8 Hz), 6.94 (1 H, dd, *J* = 8.8, 2.5 Hz), 6.82 (1 H, d, *J* = 2.5 Hz), 4.36 (2 H, q, *J* = 7.0 Hz), 3.79 (1 H, br. s.), 2.26 (3 H, s), 1.36 (3 H, t, *J* = 7.3 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.8, 140.7, 134.9, 131.6, 130.6, 122.1, 118.3, 114.8, 112.3, 37.5, 17.8, 12.9.



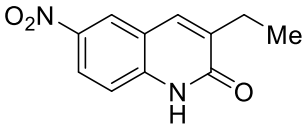
4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (130 – NI-42) Prepared according to general procedure E from **129a** (188 mg, 1.00 mmol). Purified by column chromatography (acetone:hexane ; 3:7 – 4:6) to give the title compound (129 mg, 0.37 mmol, 39%) as a colourless solid: **mp** 279 – 282 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.52 (1H, br. s), 8.03 (2H, d, *J* = 8.6 Hz, $C_{15}H$), 7.88 (2H, d, *J* = 8.6 Hz, $C_{14}H$), 7.73 (1H, s, C_4H), 7.41 (1H, d, *J* = 9.1 Hz, C_8H), 7.34 (1H, d, *J* = 2.3 Hz, C_5H), 7.23 (1H, dd, *J* = 9.0, 2.4 Hz, C_7H), 3.58 (3H, s, $C_{11}H$), 2.10 (3H, s, $C_{12}H$); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.4, 143.3, 136.3, 135.0, 133.5, 130.8, 130.0, 127.4, 123.5, 120.3, 119.9, 117.5, 115.5, 115.3, 29.4, 17.3; **HRMS**: *m/z* NSI+ [Found $(M+H)^+$ 354.908 $C_{18}H_{15}N_3O_3S$ requires $(M+H)^+$ 354.0912]; **HPLC**: Retention time (system A): t_R= 8.91 min. Purity: >95%.



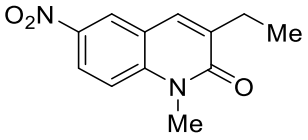
4-Cyano-*N*-(1-ethyl-3-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (131) Prepared according to general procedure E from **129b** (25 mg, 0.13 mmol). Purified by column chromagraphy (acetone:hexane ; 1-9 - 3-7) to give the title compound (22mg, 0.06 mmol, 48%) as a pale pink solid: **mp** 263 – 264 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.55 (1H, s, *NH*), 8.04 (2H, d, *J* = 8.2 Hz, $C_{16}H$), 7.89 (2H, d, *J* = 8.5 Hz, $C_{15}H$), 7.73 (1H, s, C_4H), 7.46 (1H, d, *J* = 9.1 Hz, C_8H), 7.35 (1H, d, *J* = 2.2 Hz, C_5H), 7.23 (1H, dd, *J* = 9.1, 2.5 Hz, C_7H), 4.22 (2H, q, *J* = 6.9 Hz, $C_{11}H$), 2.09 (3H, s, $C_{13}H$), 1.16 (3H, t, *J* = 6.9 Hz, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.9, 143.4, 135.1, 135.1, 133.5, 130.7, 130.0, 127.4, 123.6, 120.6, 120.0, 117.5, 115.3, 115.3, 36.9, 17.2, 12.6; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 366.0923 $C_{19}H_{17}N_3O_3S$ requires $(M-H)^-$ 366.0912]; **HPLC**: Retention time (system A): t_R= 9.40 min. Purity: >95%.



4,4-Dimethyl-2-propyl-4,5-dihydrooxazole (133) Prepared according to the method of Meyers *et al* from butyric acid (5.0 g, 61.7 mmol) to give the title compound (6.18 g, 43.8 mmol, 71%) as a colourless oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 3.91 (2H, s), 2.24 (2H, t, *J* = 7.5 Hz), 1.67 (2H, sxt, *J* = 7.4 Hz), 1.28 (6H, s), 0.98 (3H, t, *J* = 7.3 Hz). The above data is in agreement with that published previously in the literature. [125]

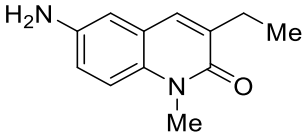


3-Ethyl-6-nitroquinolin-2(1*H*)-one (134) – Prepared by general procedure G from 2-chloro-5-nitrobenzaldehyde (2.50 g, 13.5 mmol, 1 eq.) and **133** (1.90 g, 13.5 mmol, 1 eq.) to give the title compound (2.62 g,12.0 mmol, 89%) as a brown solid: **mp** >300 °C (NMP - water); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 12.29 (1H, s), 8.64 (1H, d, *J* = 2.5 Hz), 8.28 (1H, dd, *J* = 9.1, 2.5 Hz), 7.97 (1H, s), 7.42 (1H, d, *J* = 9.1 Hz), 1.19 (3H, t, *J*=7.4 Hz) (NB Et CH_2 overlaps with DMSO peak); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 162.1, 142.1, 141.4, 137.4, 134.5, 124.0, 123.5, 118.9, 115.6, 22.8, 12.3.

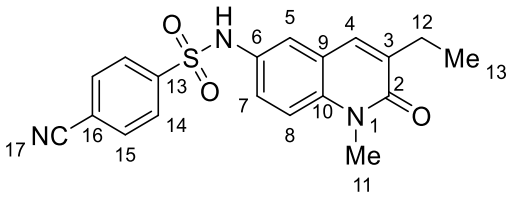


3-Ethyl-1-methyl-6-nitroquinolin-2(1*H*)-one (135) Prepared according to general procedure B from **134** (1.20 g, 5.50 mmol) to give the title compound (1.0 g, 4.34 mmol, 79%) as a brown solid,

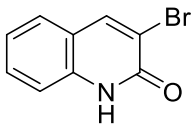
which was used without further purification **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.58 (1H, d, *J* = 2.5 Hz), 8.19 (1H, dd, *J* = 9.0, 2.5 Hz), 7.94 (1H, s), 7.41 (1H, d, *J* = 9.0 Hz), 1.18 (3H, t, *J*=7.4 Hz) *(NB Et CH_2 overlaps with* DMSO *peak)*



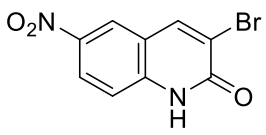
6-Amino-3-ethyl-1-methylquinolin-2(1H)-one (136) Prepared according to the procedure of Fish *et al* from **135** (500 mg, 1.03 mmol) to give the title compound (129 mg, 0.63 mmol 31%) as a black solid that was used without further purification.



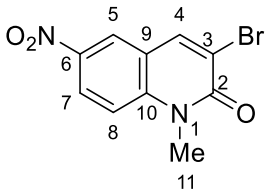
4-Cyano-*N*-(3-ethyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (137) Prepared according to general procedure E from **136** (30 mg, 0.15 mmol). Purified by column chromatography to give the title compound (30 mg, 0.08 mmol, 55% or 17% over 2 steps from **135**): **mp** 245 – 246 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.57 (1H, s, *NH*), 8.04 (2H, d, *J* = 8.6 Hz, $C_{15}H$), 7.88 (2H, d, *J* = 8.6 Hz, $C_{14}H$), 7.69 (1H, s, C_4H), 7.46 - 7.35 (2H, m, C_8H/C_5H), 7.22 (1H, dd, *J* = 8.8, 2.5 Hz, C_7H), 3.57 (3H, s, $C_{11}H$), 1.15 (3H, t, *J* = 7.5 Hz, $C_{13}H$) *NB CH_2 overlaps with* DMSO*;* **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.9, 143.3, 136.1, 135.3, 133.5, 133.5, 130.8, 127.4, 123.6, 120.4, 120.2, 117.5, 115.5, 115.3, 29.4, 23.7, 12.5; **HRMS**: *m/z* ESI- [Found (M-H)$^-$ 366.0915 $C_{19}H_{17}N_3O_3S$ requires (M-H)$^-$ 366.0912]; **HPLC**: Retention time (system A): t_R= 9.83 min. Purity: >95%.



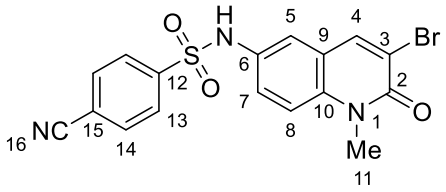
3-Bromoquinolin-2(1*H*)-one (139) Prepared by general procedure A from 3-bromoquinoline (2.5 g, 12.0 mmol) to give the title compound (2.13 g, 9.50 mmol, 79%) as a colourless solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 12.33 (1H, br. s.), 8.51 (1H, s), 7.68 (1H, d, *J* = 7.6 Hz), 7.55 (1H, t, *J* = 7.6 Hz), 7.39 (1H, d, *J* = 8.2 Hz), 7.22 (1H, t, *J* = 7.6 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6) δ ppm 157.6, 141.7, 138.1, 130.7, 127.3, 122.3, 119.4, 117.0, 115.2. The above data is in agreement with that previously reported in the literature. [126]



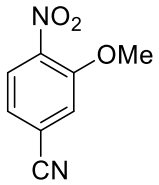
3-Bromo-6-nitroquinolin-2(1*H*)-one (140) Prepared according to general procedure C from **139** (1.0 g, 4.5 mmol) to give the title compound (615 mg, 2.3 mmol, 51%) as a pale yellow solid: **^{1}H NMR** (500 MHz, DMSO-d_6): 12.84 (1H, br. s.), 8.75 (1H, s), 8.71 (1H, d, *J* = 2.6 Hz), 8.39 – 8.33 (1H, m), 7.49 (1H, d, *J* = 8.8 Hz).



3-Bromo-1-methyl-6-nitroquinolin-2(1*H*)-one (141) Prepared by general procedure B from **140** (250 mg, 0.93 mmol). Crystallised from acetone to give the title compound (86 mg, 0.31 mmol, 33%) as a pale yellow solid; **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.78 (1H, s, C_4H), 8.76 (1H, d, *J* = 2.8 Hz, C_5H), 8.44 (1H, dd, *J* = 9.3, 2.7 Hz, C_7H), 7.78 (1H, d, *J* = 9.1 Hz, C_8H), 3.77 (3 H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 157.5, 143.1, 141.8, 140.7, 125.1, 124.0, 119.6, 118.5, 116.4, 31.5.

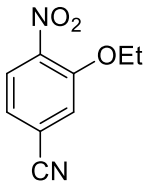


***N*-(3-bromo-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-4-cyanobenzenesulfonamide (143)** The required amine was prepared by general procedure D from **141** (40 mg, 0.14 mmol) to give crude amine which was coupled by general procedure E. Purified by column chromatography (acetone:hexane ; 2:8 -6:4) to give the title compound (28 mg, 0.07 mmol, 48% over 2 steps) as an orange solid: **mp** >300 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.64 (1H, s, *NH*), 8.52 (1H, s, C_4H), 8.03 (2H, d, *J* = 8.3 Hz, $C_{14}H$), 7.89 (2H, d, *J* = 8.4 Hz, $C_{13}H$), 7.50 – 7.46 (2H, m, C_8H/ C_5H), 7.33 (1H, dd, *J* = 9.1, 2.5 Hz, C_7H), 3.64 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 157.0, 143.2, 140.4, 136.5, 133.5, 131.4, 127.4, 125.0, 120.3, 119.8, 117.5, 117.4, 116.3, 115.4, 30.9; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 415.9711, $C_{17}H_{12}N_3O_3SBr$ requires $(M-H)^-$ 415.9705]; **HPLC**: Retention time (system A): t_R= 9.39 min. Purity: >95%.

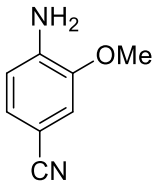


3-Methoxy-4-nitrobenzonitrile (145a) 3-fluoro-4-nitrobenzonitrile (5.00 g, 30.1 mmol) was dissolved in dry THF and cooled to 0 °C. Sodium methoxide (4.87 g, 90.3 mmol, 3 eq.) was added in one portion with vigorous stirring and the resulting suspension was stirred at 0 °C for 3 hours, neutralised with HCl (2M), and washed with EtOAc (3 × 50 mL). The organic fractions were pooled, dried over *anhydrous* $MgSO_4$, filtered and concentrated under reduced pressure to give the title

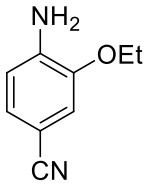
compound (5.65 g, 30.1 mmol, quant) as a pale yellow solid: **mp** 122 - 123 °C (ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.90 (1H, d, *J* = 8.8 Hz), 7.41 - 7.34 (2H, m), 4.04 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 152.7, 142.4, 126.1, 124.2, 117.4, 117.1, 116.9, 57.0. The above data is in agreement with that previously reported in the literature. [127]



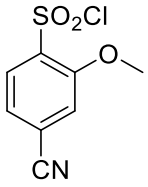
3-Ethoxy-4-nitrobenzonitrile (145b) Prepared analogously to compound **145a** from 3-fluoro-4-nitrobenzonitrile (3.00 g, 18.1 mmol) and sodium ethoxide (3.69 g, 54.2 mmol, 3 eq.) to give the title compound (3.02 g, 15.7 mmol, 87%) as an orange solid: **mp** 121 - 123 °C (ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.86 (1H, d, *J* = 8.8 Hz), 7.38 - 7.33 (2H, m), 4.24 (2H, q, *J* = 6.9 Hz), 1.53 (3H, t, *J* = 6.9 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 152.0, 142.8, 125.8, 123.9, 117.9, 117.1, 116.9, 66.2, 14.3.



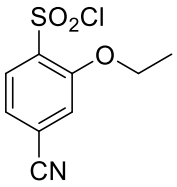
4-Amino-3-methoxybenzonitrile (146a) Prepared by general procedure M from **145a** (5.00 g, 28.0 mmol). Purified by column chromatography (EtOAc:Hexane ; 3:7) to give the title compound (20.8 mmol, 3.08 g, 74%) as a dark red solid; **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.15 (1H, dd, *J* = 8.0, 1.4 Hz), 6.99 (1H, s), 6.68 (1H, d, *J* = 7.9 Hz), 4.31 (2H, br. s.), 3.90 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 146.2, 141.1, 126.7, 120.3, 113.6, 113.0, 99.6, 55.5. The above data is in agreement with that previously reported in the literature. [128]



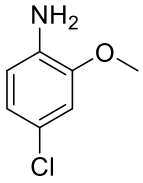
4-Amino-3-ethoxybenzonitrile (146b) Prepared by general procedure N from **145b** (2.5 g, 13.02 mmol) to give the title compound (910 mg, 5.60 mmol, 43%) as an orange solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.12 (1H, dd, *J* = 8.2, 1.6 Hz), 6.96 (1H, d, *J* = 1.6 Hz), 6.68 (1H, d, *J* = 8.2 Hz), 4.34 (2H, br. s.), 4.08 (2H, d, *J* = 6.9 Hz), 1.47 (3H, t, *J* = 6.9 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 145.5, 141.1, 126.5, 120.4, 113.8, 113.6, 99.6, 64.2, 14.7.



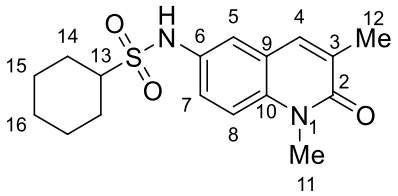
4-Cyano-2-methoxybenzenesulfonyl chloride (148) Prepared by general procedure O from **146a** (500 mg, 3.38 mmol) to give a crude solid of the title compound (480 mg) as an orange solid: Relevant **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.11 (1H, d, *J* = 8.2 Hz), 7.49 (1H, d, *J* = 8.2 Hz), 7.41 (1H, s), 4.15 (3H, s).



4-Cyano-2-ethoxybenzenesulfonyl chloride (149) Prepared by general procedure O from **146b** (1.0 g, 6.2 mmol) to give a crude solid of the title compound (417 mg) as an orange solid Relevant **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.09 (1H, d, *J* = 8.2 Hz), 7.40 (1H, dd, *J* = 8.2, 1.6 Hz), 7.38 (1H, s), 4.35 (2H, q, *J* = 7.0 Hz), 1.61 (3H, t, *J* = 6.9 Hz)

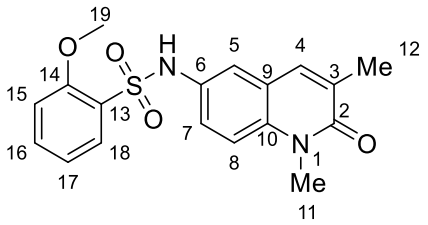


4-Chloro-2-methoxyaniline (151) Prepared according to the procedure of Diab *et al* from 2-methoxy-4-chloro-nitroaniline (1.0 g, 5.33 mmol). Purified by column chromatography (ethyl acetate:hexane ; 1:1) to give the title compound (660 mg, 4.21 mmol, 79%) as a dark red oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 6.75 - 6.81 (2H, m), 6.61 - 6.67 (1H, m), 3.86 (3H, s), 3.78 (2H, br. s.); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 147.5, 134.8, 122.7, 120.7, 115.2, 111.1, 55.7. The above data is in agreement with that published previously in the literature[129]

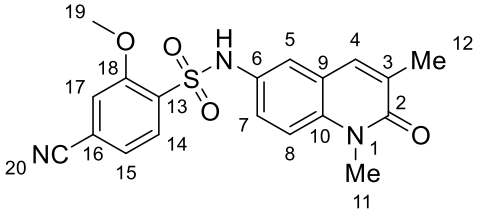


***N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)cycloHexaneulfonamide (152)** Prepared according to general procedure E from **129a** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 1:19 – 1:9) to give the title compound (28 mg, 0.08 mmol, 79%) as a colorless solid: **mp** 158 - 160 °C (acetone-hexane); **mp** 177 - 178 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 9.81 (1H, s, *NH*), 7.78 (1H, s, C_4H), 7.51 – 7.26 (3H, m, C_5H/ C_8H/ C_7H), 3.62 (3H, s, $C_{11}H$), 3.00 – 2.91 (1H, m), 2.12 (3H, s, $C_{12}H$), 2.02 (2H, d, *J* = 11.4 Hz), 1.74 (2H, d, *J* =

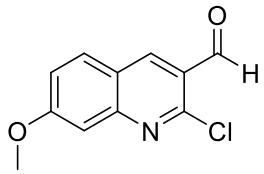
10.6 Hz), 1.57 (1H, d, *J* = 13.9 Hz), 1.41 (2H, m), 1.27 - 1.04 (3H, m); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.4, 135.5, 135.2, 132.6, 129.8, 122.3, 120.4, 118.0, 115.5, 58.7, 29.4, 26.0, 24.7, 24.3, 17.3; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 333.1272 $C_{17}H_{22}N_2O_3S$ requires $(M-H)^-$ 333.1273]; **HPLC**: Retention time (system A): t_R= 9.37 min. Purity: >95%.



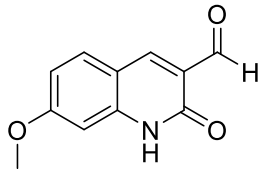
***N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (153)**
Prepared according to general procedure E from **129a** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 1:9) to give the title compound (25 mg, 0.07 mmol, 65%) as a yellow solid: **mp** 221 - 222 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 9.97 (1H, s, *NH*), 7.72 (1H, dd, *J* = 7.8, 1.8 Hz, *C$_{18}$H*), 7.67 (1H, s, *C$_4$H*), 7.57 - 7.50 (1H, m, *C$_{16}$H*), 7.35 (1H, d, *J* = 9.1 Hz, *C$_8$H*), 7.31 - 7.25 (2H, m, *C$_5$H/C$_7$H*), 7.16 (1H, d, *J* = 7.8 Hz, *C$_{17}$H*), 6.99 (1 H, td, *J* = 7.6, 1.0 Hz, *C$_{15}$H*), 3.91 (3H, s), 3.54 (3H, s), 2.07 (3H, d, *J* = 1.0 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.4, 156.3, 135.6, 135.0, 135.0, 131.9, 130.2, 129.8, 126.1, 122.5, 120.1, 120.0, 118.4, 115.2, 112.7, 56.0, 29.3, 17.2; **HRMS**: *m/z* FTMS- [Found $(M-H)^-$ 357.0921 $C_{18}H_{18}N_2O_4S$ requires M^- 357.0909]; **HPLC**: Retention time (system A): t_R= 8.56 min. Purity: >95%.



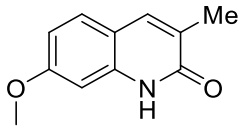
4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (154 – NI-57) Prepared according to general procedure E from **129a** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 4:6) to give the title compound (9 mg, 0.02 mmol, 24%) as a colourless solid: **mp** 246 – 247 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 10.26 (1H, s, *NH*), 7.86 (1H, d, *J* = 8.1 Hz, *C$_{14}$H*), 7.73 (1H, d, *J* = 1.3 Hz, *C$_{17}$H*), 7.70 (1H, s, *C$_4$H*), 7.47 (1H, dd, *J* = 8.1, 1.3 Hz, *C$_{15}$H*), 7.37 (1H, d, *J* = 9.1 Hz, *C$_8$H*), 7.30 (1H, d, *J* = 2.5 Hz, *C$_5$H*), 7.26 (1H, dd, *J* = 9.1, 2.5 Hz, *C$_7$H*), 3.98 (3H, s, *C$_{19}$H*), 3.56 (3H, s, *C$_{11}$H*), 2.08 (3H, d, *J* = 1.0 Hz, *C$_{12}$H*); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.3, 156.3, 135.6, 135.0, 135.0, 131.9, 130.2, 129.8, 126.1, 122.5, 120.1, 120.1, 118.4, 115.1, 112.7, 56.1, 29.3, 17.3; **HRMS**: *m/z* NSI+ [Found $(M+H)^+$ 384.1013 $C_{19}H_{17}N_3O_4S$ requires $(M+H)^+$ 384.0940]; **HPLC**: Retention time (system A): t_R= 6.04 min. Purity: >95%.



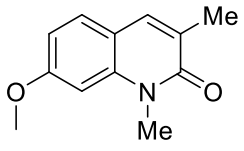
7-Methoxyquinoline-3-carbaldehyde (155) Prepared according to the procedure of Meth-Cohn *et al* starting from **94** (6 g, 36.3 mmol) to give the title compound (4.82 g, 25.8 mmol, 71%) as a pale yellow solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 10.53 (1H, s), 8.68 (1H, s), 7.87 (1H, d, *J* = 9.1 Hz), 7.40 (1H, d, *J* = 2.5 Hz), 7.29 (2H, dd, *J* = 9.1, 2.5 Hz), 4.01 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 189.1, 164.2, 152.0, 151.1, 139.5, 130.8, 124.4, 121.8, 121.6, 106.3, 55.9. The above data is in agreement with that previously reported in the literature. [94]



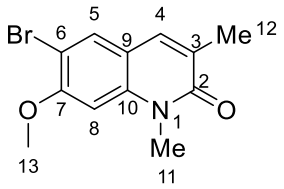
7-Methoxy-2-oxo-1,2-dihydroquinoline-3-carbaldehyde (156) 155 (3.0 g, 13.5 mmol, 1 eq.) was suspended in HCl (6.0 M, 45 mL) and heated at reflux for 6 hours. The suspension was allowed to cool to RT and filtered. The precipitate was washed with H_2O and dried to give the title compound (1.76 g, 8.68 mmol, 64%) as a brown solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 12.01 (1H, s), 8.43 (1H, s), 7.78 – 7.71 (1H, m), 7.66 (1H, d, *J* = 8.8 Hz), 6.90 - 6.82 (2H, m), 3.85 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 162.3, 161.1, 141.5, 141.0, 130.4, 126.8, 122.6, 113.5, 111.8, 97.5, 55.5.



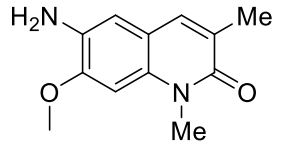
7-Methoxy-3-methylquinolin-2(1*H*)-one (157) To a solution of **156** (1.50 g, 7.39 mmol, 1 eq.) in TFA (15 mL) at 0 °C was added triethylsilane (2.57 g, 22.2 mmol, 3 eq.) dropwise. The solution was allowed to warm to RT, stirred overnight and then poured onto ice (~250 g). The resulting precipitate was filtered, washed with H_2O, triturated with Et_2O and dried to provide the title compound (796 mg, 4.21 mmol, 57%) as a pale yellow solid: **mp** 180 – 181°C (TFA-water); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 11.60 (1H, br. s.), 7.68 (1H, s), 7.49 (1H, d, *J* = 8.5 Hz), 6.81 - 6.75 (2H, m), 3.79 (3H, s), 2.05 (3H, d, *J* = 0.9 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 162.7, 160.1, 139.5, 136.2, 128.3, 126.3, 113.6, 110.2, 97.9, 55.2, 16.3.



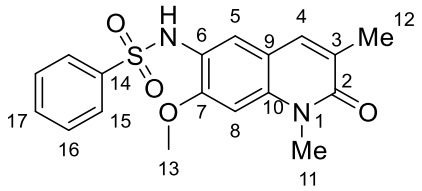
7-Methoxy-1,3-dimethylquinolin-2(1*H*)-one (158) Prepared according to general procedure B from **157** (750 mg, 3.97 mmol). Purified by column chromatography (acetone:hexane ; 1:9) to provide the title compound (652 mg, 3.21 mmol, 81%) as a colourless solid: **mp** 144 - 145 °C (acetone-hexane); **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.40 (1H, s), 7.33 (1H, d, *J* = 8.6 Hz), 6.77–6.67 (2H, m), 3.84 (3H, s), 3.64 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 163.3, 160.7, 140.6, 135.5, 129.1, 126.7, 115.0, 109.4, 98.5, 55.6, 29.7, 17.2.



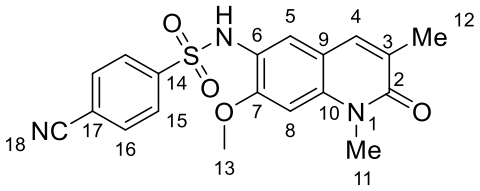
6-Bromo-7-methoxy-1,3-dimethylquinolin-2(1*H*)-one (159) 158 (812 mg, 4 mmol 1 eq.) was dissolved in DMF (4 mL) and *N*-bromosuccinimide (855 mg, 4.8 mmol, 1.2 eq.) was added in one portion. The solution was stirred overnight at RT. Cold water (30 mL) was added and the resulting precipitate was filtered off to give the title compound (826 mg, 2.93 mmol, 73%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.68 (1H, s, *C_4H*), 7.40 (1H, s, *C_5H*), 6.75 (1H, s, *C_8H*), 4.03 (3H, s, *$C_{13}H$*), 3.75 (3H, s, *$C_{11}H$*), 2.25 (3H, s, *$C_{12}H$*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.9, 156.5, 139.8, 134.4, 131.7, 128.0, 115.6, 105.7, 97.0, 56.5 , 29.9, 17.5.



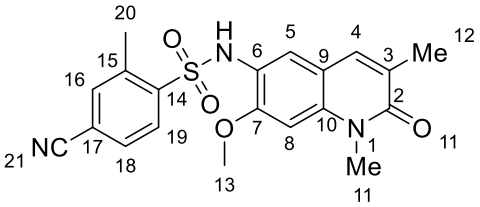
6-Amino-7-methoxy-1,3-dimethylquinolin-2(1*H*)-one (160) Prepared by general procedure J from **159** (500 mg, 1.77 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 6:4) to give the title compound as a solution in NMP that was used without further purification.



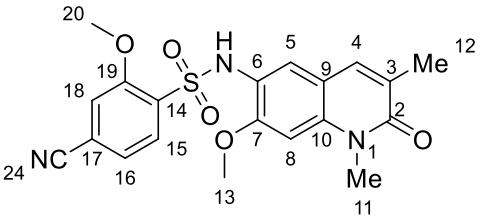
***N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (161)** Prepared by general procedure E from a solution of **160** in NMP (38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 3:7) to give the title compound (36 mg, 0.10 mmol, 57% over 2 steps) as a colourless solid: **^{1}H NMR** (500 MHz, DMSO-*d_6*): δ ppm 9.60 (1H, br. s, *NH*), 7.72–7.66 (3H, m, *$C_{15}H/C_4H$*), 7.63–7.59 (1H, m, *$C_{17}H$*), 7.55–7.47 (3H, m, *$C_{16}H/ C_5H$*), 6.81 (1H, s, *C_8H*), 3.60 (3H, s, *$C_{13}H$*), 3.57 (3H, s, *$C_{11}H$*), 2.08 (3H, s, *$C_{12}H$*); **^{13}C NMR** (126 MHz, DMSO-*d_6*): δ ppm 161.8, 154.4, 140.4, 138.4, 135.2, 132.5, 128.7, 126.6, 126.1, 125.2, 120.2, 113.2, 97.4, 55.7, 29.6, 17.1; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 357.0920, $C_{18}H_{18}N_2O_4S$ requires $(M-H)^-$ 357.0909]; **HPLC**: Retention time (system A): t_R= 9.02 min. Purity: >95%.



4-Cyano-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (162) Prepared by general procedure E from a solution of **160** in NMP (38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 6:4) to give the title compound (35 mg, 0.09 mmol, 49% over 2 steps) as a pale pink solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.98 (1H, br. s, *NH*), 8.03 (2H, d, *J* = 8.5 Hz, $C_{15}H$), 7.82 (2H, d, *J* = 8.2 Hz, $C_{16}H$), 7.74 (1H, s, C_4H), 7.51 (1H, s, C_5H), 6.81 (1H, s, C_8H), 3.61 (3H, s, $C_{13}H$), 3.53 (3H, s, $C_{11}H$), 2.09 (3H, s, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.8, 154.7, 144.8, 138.8, 135.2, 133.0, 127.4, 126.5, 126.2, 119.4, 117.8, 114.8, 113.3, 97.5, 55.6, 29.6, 17.1; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 382.0862, $C_{19}H_{17}N_3O_4S$ requires $(M-H)^-$ 382.0862]; **HPLC**: Retention time (system A): t_R= 9.19 min. Purity: >95%.

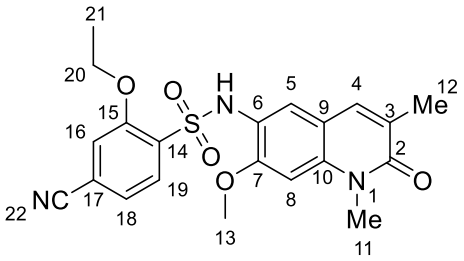


4-Cyano-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methylbenzenesulfonamide (163) Prepared by general procedure E from a solution of **160** in NMP (38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 4:6) to give the title compound (26 mg, 0.07 mmol, 37% over 2 steps) as a pale yellow solid: **mp** 274 – 275 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.97 (1H, s, *NH*), 7.95 (1H, s, $C_{16}H$), 7.74 - 7.66 (3H, m, $C_4H/C_{18}H/C_{19}H$), 7.50 (1H, s, C_5H), 6.78 (1H, s, C_8H), 3.59 (3H, s, $C_{13}H$), 3.52 (3H, s, $C_{11}H$), 2.70 (3H, s, $C_{20}H$), 2.08 (3H, s, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.8, 155.1, 142.9, 139.0, 138.8, 135.6, 135.1, 129.6, 129.4, 127.2, 126.2, 118.9, 117.7, 114.7, 113.3, 97.4, 55.5, 29.6, 19.5, 17.1; **HRMS**: *m/z* ESI- [Found $(M+CO_2^-)^-$ 441.0743, $C_{20}H_{19}N_3O_4S$ requires $(M+CO_2^-)^-$ 441.0995]; **HPLC**: Retention time (system A): t_R= 11.04 min. Purity: >95%.

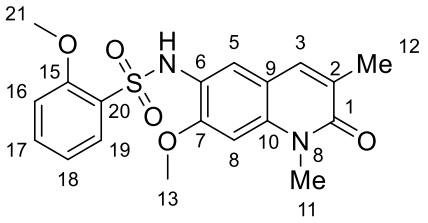


4-Cyano-2-methoxy-N-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (164) Prepared by general procedure E from a solution of **160** in NMP (114 mg, 0.54 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 1:1) to give the title compound (81 mg, 0.20 mmol, 37% over 2 steps) as an off-white solid: **mp** 259 – 261 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 9.35 (1H, br. S, *NH*), 7.76 (1H, s, C_4H), 7.70

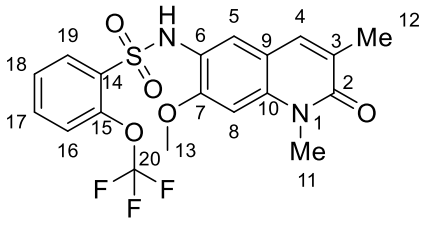
(2H, m, $C_{15/18}H$), 7.46 (1H, s, C_5H), 7.43 (1H, dd, J = 8.0, 1.4 Hz, $C_{16}H$), 6.81 (1H, s, C_8H), 3.97 (3H, s, $C_{20}H$), 3.65 (3H, s, $C_{13}H$), 3.60 (3H, s, $C_{11}H$), 2.07 (3H, d, J = 0.8 Hz, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.8, 156.7, 154.6, 138.5, 135.1, 132.4, 130.0, 126.1, 126.0, 123.6, 119.8, 117.7, 116.3, 116.2, 113.2, 97.3, 56.9, 55.9, 29.5, 16.8; **HRMS**: *m/z* NSI+[Found $(M+H)^+$ 414.1113, $C_{20}H_{19}N_3O_5S$ requires $(M-H)^-$ 414.1112]; **HPLC**: Retention time (system A): t_R= min. Purity: >95%.



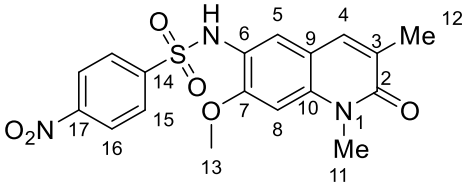
4-Cyano-2-ethoxy-N-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (165) Prepared by general procedure E from a solution of **160** in NMP (38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 1:1) to give the title compound (16 mg, 0.04 mmol, 22% over 2 steps) as an orange solid: **mp** 220 – 223 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6) δ ppm 9.05 (1H, s, *NH*), 7.75 (1H, d, J = 1.1 Hz, $C_{16}H$), 7.74 (1H, d, J = 8.1 Hz, $C_{19}H$), 7.71 (1H, s, C_4H), 7.50 (1H, s, C_5H), 7.43 (1H, dd, J = 8.0, 1.3 Hz, $C_{18}H$), 6.84 (1H, s, C_8H), 4.26 (2H, q, J = 7.0 Hz, $C_{20}H$), 3.67 (3H, s, $C_{13}H$), 3.61 (3H, s, $C_{11}H$), 2.07 (3H, d, J = 0.8 Hz, $C_{12}H$), 1.35 (4H, t, J = 7.0 Hz, $C_{21}H$) ; **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.8, 156.0, 154.2, 138.3, 135.1, 132.3, 130.3, 126.2, 125.0, 123.5, 120.0, 117.7, 116.9, 116.4, 113.2, 97.3, 65.3, 55.9, 29.6, 17.1, 14.0; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 426.1133, $C_{21}H_{21}N_3O_5S$ requires $(M-H)^-$ 426.1124]; **HPLC**: Retention time (system A): t_R= 10.44 min. Purity: >95%.



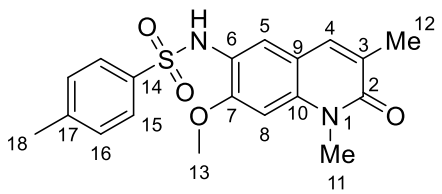
2-Methoxy-N-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (166) Prepared by general procedure E from a solution of **160** in NMP (38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 3:7) to give the title compound (35 mg, 0.09 mmol, 51% over 2 steps) as a colourless solid: **mp** 246 - 247 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.82 (1H, s, *NH*), 7.67 (1H, s, C_4H), 7.61 - 7.53 (2H, m, $C_{19}H$/ $C_{17}H$), 7.48 (1H, s, C_5H), 7.21 (1H, d, J = 7.8 Hz, $C_{16}H$), 6.95 (1H, td, J = 7.6, 1.0 Hz, $C_{18}H$), 6.83 (1H, s, C_8H), 3.92 (3H, s, $C_{21}H$), 3.73 (3H, s, $C_{13}H$), 3.59 (3H, s, $C_{11}H$), 2.06 (3H, d, J = 1.0 Hz, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.7, 156.6, 153.5, 137.8, 135.2, 134.8, 129.5, 127.1, 126.1, 123.5, 120.8, 119.6, 113.1, 112.5, 97.2, 56.1, 56.1, 29.5, 17.1; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 387.1024, $C_{19}H_2N_2O_5S$ requires $(M-H)^-$ 387.1014]; **HPLC**: Retention time (system A): t_R= 9.33 min. Purity: >95%.



***N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-(trifluoromethoxy)benzenesulfonamide (167)** Prepared by general procedure E from a solution of **160** in NMP (38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 2:8) to give the title compound (38 mg, 0.09 mmol, 49% over 2 steps) as a pale yellow solid: **mp** 189 – 190 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.69 (1H, br. s), 7.78-7.72 (2H,m , $C_{19}H$, $C_{17}H$), 7.71(1H, s, C_4H), 7.56 (1H, d, J = 7.6 Hz, $C_{16}H$), 7.49 (1H, s, C_5H), 7.44 (1H, t, J = 7.6 Hz, $C_{18}H$), 6.81 (1H, s, C_8H), 3.61 (3H, s, $C_{13}H$), 3.56 (3H, s, $C_{11}H$), 2.07 (3H, s, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.2, 154.9, 145.3, 145.3, 138.7, 135.1, 134.8, 132.6, 130.4, 126.8, 126.6, 126.1, 120.4, 120.9 (q, J = 90.7 Hz), 113.2, 97.4, 55.6, 29.6, 17.0; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 441.0734, $C_{19}H_{17}N_2O_5SF_3$ requires $(M-H)^-$ 441.0732]; **HPLC**: Retention time (system A): t_R= 11.02 min. Purity: >95%.

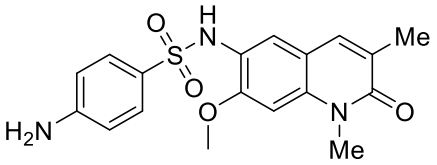


***N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-4-nitrobenzenesulfonamide (168)** Prepared by general procedure E from a solution of **160** in NMP (76 mg, 0.36 mmol). Purified by column chromatography (acetone:hexane ; 1:9 – 1:4) to give the title compound (94 mg, 0.23 mmol, 66% over 2 steps) as a bright yellow solid: **mp** 260 - 262 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.06 (1H, s, *NH*), 8.37 (2H, d, J = 8.8 Hz, $C_{16}H$), 7.91 (2H, d, J = 8.8 Hz, $C_{15}H$), 7.74 (1H, s, C_4H), 7.51 (1H, s, C_5H), 6.82 (1H, s, C_8H), 3.61 (3H, s, $C_{13}H$), 3.52 (3H, s, $C_{11}H$), 2.09 (3H, s, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.8, 154.8, 149.5, 146.2, 138.9, 135.2, 128.2, 126.6, 126.2, 124.1, 119.2, 113.3, 97.5, 55.4, 29.4, 17.2; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 402.0768, $C_{18}H_{17}N_3O_6S$ requires $(M-H)^-$ 402.0760]; **HPLC**: Retention time (system A): t_R= 10.98 min. Purity: >95%.

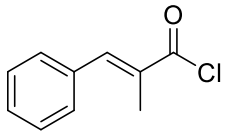


***N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-4-methylbenzenesulfonamide (169):** Prepared by general procedure E from a solution of **160** in NMP (38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 1:9 – 3:7) to give the title compound (40 mg,

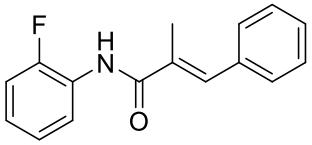
0.11 mmol, 60% over 2 steps) as a pale yellow solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.51 (1H, s, *NH*) 7.71 (1H, s, C_4H) 7.58 (2H, d, *J* = 8.2 Hz, $C_{15}H$) 7.48 (1H, s, C_5H) 7.32 (2H, d, *J* = 8.2 Hz, $C_{16}H$) 6.82 (1H, s, C_8H) 3.60 (3H, s, $C_{13}H$), 3.54 (3H, s, $C_{11}H$), 2.35 (3H, s, $C_{18}H$) 2.08 (3H, s, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.8, 154.1, 142.7, 138.2, 137.6, 135.2, 129.2, 126.7, 126.1, 124.6, 120.5, 113.2, 97.5, 55.8, 29.6, 20.9, 17.1; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 371.1070, $C_{19}H_{20}N_2O_4S$ requires $(M-H)^-$ 371.1066]; **HPLC**: Retention time (system A): t_R= 9.06 min. Purity: >95%.



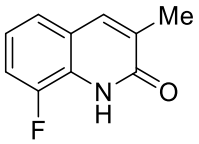
4-Amino-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (25) 168 (25 mg, 0.06 mmol, 1 eq.) and palladium on carbon (5% by weight, 13 mg, 0.006 mmol, 0.1 eq.) were placed in a sealed rbf and backfilled with nitrogen 2 times and then EtOH (40 mL) was added. The flask was fitted with a balloon of hydrogen and purged and then backfilled 5 times. The resulting suspension was stirred at RT overnight, purged with nitrogen for 20 mins and then filtered through a pad of Celite ® eluting with EtOH. The filtrate was concentrated under reduced pressure to give the title compound (17 mg, 0.05 mmol, 75%) as a beige solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.98 (1H, s), 7.69 (1H, s), 7.33 (2H, d, *J* = 8.5 Hz), 6.84 (1H, s), 6.50 (2H, d, *J* = 8.5 Hz), 5.92 (1H, s), 3.72 (3H, s), 3.63 (3H, s), 2.08 (3H, s).



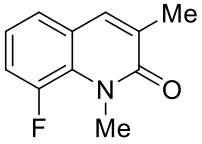
(*E*)-2-methyl-3-phenylacryloyl chloride (172) Prepared according to the procedure of Aggarwaal *et al* starting from (E)-2-methyl-3-phenylacrylic acid (), oxalyl chloride () and DMF () to give the title compound (19 % DMF by ^{1}H NMR) which was used without further purification in the next step. Relevant **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 8.06 (1H, d, *J* = 1.3 Hz), 7.51 - 7.40 (5H, m), 2.22 (3H, d, *J* = 1.3 Hz). The above data is in agreement with that previously reported in the literature. [130]



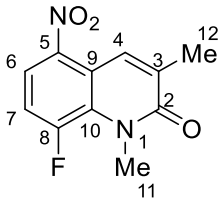
(*E*)-*N*-(2-fluorophenyl)-2-methyl-3-phenylacrylamide (173) Prepared by general procedure K from 2-fluoroaniline (2.5 g, 22.5 mmol) to give the title compound (5.05 g, 19.8 mmol, 88%) as a colourless solid which was used without further purification.



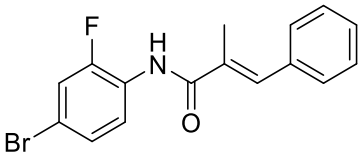
8-Fluoro-3-methylquinolin-2(1*H*)-one (174) Prepared by general procedure L from **173** (5 g, 19.6 mmol) to give the title compound (2.91 g, 16.47 mmol, 84%) as a pale pink solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 11.73 (1H, br. s.), 7.81 (1H, s), 7.41 (1H, d, *J* = 7.8 Hz), 7.32 (1H, dd, *J* = 10.7, 8.5 Hz), 7.17 - 7.08 (1H, m), 2.11 (3H, s); **mp** 199 - 202 °C (chlorobenzene-water);**^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 162.1, 147.9 (d, *J* = 244.7 Hz), 135.9 (d, *J* = 1.8 Hz), 131.3, 126.3 (d, *J* = 13.7 Hz), 122.7 (d, *J* = 2.7 Hz), 121.6 - 121.4 (2C signals), 114.3 (d, *J* = 17.4 Hz), 16.6.



8-Fluoro-1,3-dimethylquinolin-2(1*H*)-one (175) Prepared by general procedure B from **174** (1.0 g, 5.65 mmol). Purified by column chromatography (acetone:hexane 1:9) to give the title compound (410 mg, 2.15 mmol, 38%) as a pale pink solid: **mp** 104 - 105 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 7.76 (1H, s), 7.43 (1H, d, *J* = 7.6 Hz), 7.37 (1H, dd, *J* = 15.1, 7.9 Hz), 7.20 (1H, d, *J* = 4.1 Hz), 3.78 (3H, d, *J* = 8.2 Hz), 2.11 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 163.2, 150.1 (d, *J* = 245.6 Hz), 135.4 (d, *J* = 2.7 Hz), 131.0, 128.2 (d, *J* = 6.4 Hz), 123.7 (d, *J* = 3.7 Hz), 123.5 (d, *J* = 2.7 Hz), 122.3 (d, *J* = 8.2 Hz), 116.6 (d, *J* = 23.8 Hz), 33.5 (d, *J* = 15.6 Hz), 17.7.

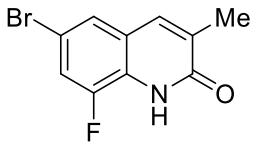


8-Fluoro-1,3-dimethyl-5-nitroquinolin-2(1*H*)-one (176) Prepared by general procedure C from **175** (350 mg, 1.81 mmol). Residue crystallised from EtOH to give the title compound (307mg, 1.30 mmol, 71%) as yellow needles: **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.06 - 8.02 (1H, m, C_3H), 7.91 (1H, dd, *J* = 8.8, 3.8 Hz, C_6H), 7.62 (1H, dd, *J* = 14.0, 9.0 Hz, C_7H), 3.83 (3H, d, *J* = 9.6 Hz, $C_{11}H$), 2.20 (3H, d, *J* = 1.3 Hz, $C_{12}H$); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.9, 152.0 (d, *J* = 254.8 Hz), 143.0, 135.1, 129.4 (d, *J* = 7.3 Hz), 129.0, 119.6 (d, *J* = 9.2 Hz), 117.0, 115.6 (d, *J* = 26.6 Hz), 34.0 (d, *J* = 16.5 Hz), 18.4.

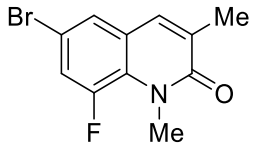


(*E*)-*N*-(4-bromo-2-fluorophenyl)-2-methyl-3-phenylacrylamide (178) Prepared by general procedure K from 2-fluro-4-bromoaniline (2.50 g, 13.2 mmol) to give the title compound (4.32 g,

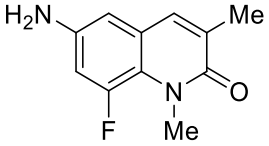
12.94 mmol, 98%) as a colourless solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.81 (1H, s), 7.65 - 7.55 (2H, m), 7.50 - 7.33 (7H, m), 2.12 (3H, d, *J* = 1.3 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 167.9, 155.3 (d, *J* = 253.9 Hz), 135.7, 134.1, 131.8, 129.4, 128.5, 128.0, 127.9 (d, *J* = 1.8 Hz), 127.3 (d, *J* = 3.7 Hz), 125.7 (d, *J* =11.9 Hz), 119.1 (d, *J* = 23.8 Hz), 117.1 (d, *J* = 9.2 Hz), 14.2.



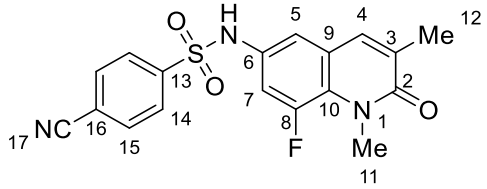
6-Bromo-8-fluoro-3-methylquinolin-2(1H)-one (179) Prepared by general procedure L from **178** (3.5 g, 10.5 mmol). The title compound (443 mg, 1.73 mmol, 17 % yield) was isolated as a waxy red solid which was used without further purification in the next step: Relevant **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 11.91 (1H, br. s.), 7.78 (1H, t, *J* = 1.3 Hz), 7.68 (1H, t, *J* = 2.0 Hz), 7.62 (1H, dd, *J* = 10.4, 2.0 Hz), 2.11 (3H, d, *J* = 1.0 Hz).



6-Bromo-8-fluoro-1,3-dimethylquinolin-2(1*H*)-one (180) Prepared by general procedure B from **179** (430 mg, 1.68 mmol) to give the title compound (112 mg, 0.41 mmol, 25 %) as a dark red solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.43 (1H, t, *J*=1.8 Hz), 7.42 - 7.40 (1H, m), 7.36 (1H, dd, *J* = 13.6, 2.3 Hz), 3.92 (3H, d, *J* = 8.1 Hz), 2.27 (3H, d, *J* = 1.0 Hz).

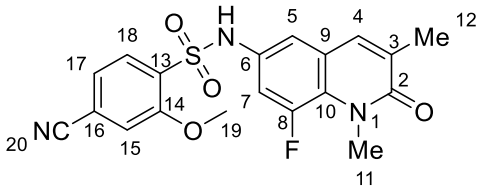


6-Amino-8-fluoro-1,3-dimethylquinolin-2(1*H*)-one (181) Prepared by general procedure J from **180** (75 mg, 0.28 mmol). Purified by column chromatography (acetone:hexane ; 3:7 – 4:6) to give a solution of the title compound in NMP which was used without further purification in the next step.

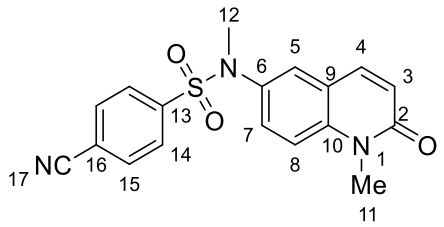


4-Cyano-N-(8-fluoro-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (182) Prepared by general procedure E from **181** (29 mg, 0.14 mg). Purified by column chromatography (acetone:hexane ; 3:7 -6:4) to give the title compound (11 mg, 0.03 mmol, 21% over 2 steps) as a colourless solid: **mp** 265 – 266 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.04 (2H, d, *J* = 8.1 Hz, $C_{14}H$), 7.92 (2H, d, *J* = 8.3 Hz, $C_{15}H$), 7.73 (1H, s, C_4H), 7.13 (1H, d, *J* = 0.8 Hz, C_5H), 7.08 (1H, dd, *J* = 15.7, 2.0 Hz, C_7H), 3.72 (3H, d, *J* = 8.3 Hz, $C_{11}H$), 2.09 (3H, s, $C_{12}H$); **^{13}C**

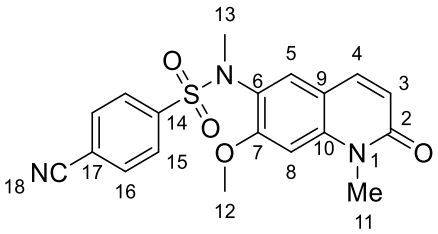
NMR (126 MHz, DMSO-d_6): δ ppm 161.6, 149.1 (d, *J* = 246.5 Hz), 143.1 (d, *J* = 1.8 Hz), 135.1, 133.6, 131.0, 127.7, 124.9 (d, *J* = 7.3 Hz), 123.0 (d, *J* = 3.7 Hz), 117.5, 115.5, 115.1 (d, *J* = 2.7 Hz), 110.1 (d, *J* = 26.6 Hz), 32.7 (d, *J* = 14.7 Hz), 17.2; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 370.0659, $C_{18}H_{14}N_3O_3SF$ requires $(M-H)^-$ 370.0662]; **HPLC**: Retention time (system A): t_R= 8.68 min. Purity: >95%.



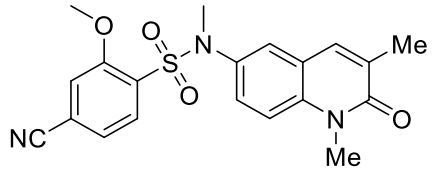
4-Cyano-*N*-(8-fluoro-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (183) Prepared by general procedure E from **181** (29 mg, 0.14 mg). Purified by column chromatography (acetone:hexane ; 2:8) to give the title compound (7 mg, 0.02 mmol, 13% over 2 steps) as a colourless solid: **mp** 214 -215 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6) δ ppm 10.54 (1H, s), 7.94 (1H, d, *J* = 8.0 Hz, $C_{18}H$), 7.75 (1H, d, *J* = 1.1 Hz, $C_{15}H$), 7.72 (1H, s, C_4H), 7.52 (1H, dd, *J* = 8.1, 1.3 Hz, $C_{17}H$), 7.12 (1H, d, *J* = 2.3 Hz, C_5H), 7.09 (1H, dd, *J* = 16.0, 2.4 Hz, C_7H), 3.71 (3H, d, *J* = 8.1 Hz, $C_{11}H$), 2.08 (3H, s, $C_{12}H$) **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.6, 156.3, 149.0 (d, *J* = 246.5 Hz), 135.1, 131.7, 131.6, 131.1, 130.7 (d, *J* = 58.7 Hz), 124.5 (d, *J* = 6.4 Hz), 124.2, 122.9 (d, *J* = 3.7 Hz), 117.4, 117.1, 116.9, 114.2 (d, *J* = 2.7 Hz), 109.4 (d, *J* = 27.5 Hz), 57.0, 32.7 (d, *J* = 14.7 Hz), 17.2; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 400.0765, $C_{19}H_{16}N_3O_4SF$ requires $(M-H)^-$ 400.0767]; **HPLC**: Retention time (system A): t_R= 9.95 min. Purity: >95%.



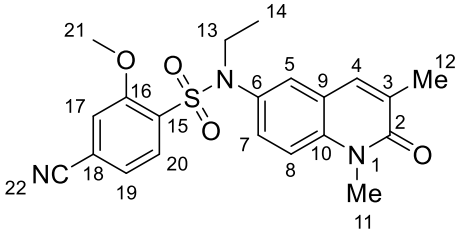
4-Cyano-*N*-methyl-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (184) Prepared by general procedure H from **23** (50 mg, 0.15 mmol). Purified by column chromatography (acetone:hexane ; 3:7) to give the title compound (33 mg, 0.09 mmol, 63%) as a colourless solid: **mp** 217 – 218 °C (DCM); **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 8.08 (2H, d, *J* = 8.3 Hz, $C_{15}H$), 7.85 (1H, d, *J* = 9.6 Hz, C_4H), 7.70 (2H, d, *J* = 8.6 Hz, $C_{14}H$), 7.54 - 7.49 (2H, m, C_5H/ C_8H), 7.37 (1H, dd, *J* = 9.1, 2.5 Hz, C_7H), 6.65 (1H, d, *J* = 9.6 Hz, C_3H), 3.61 (3H, s, $C_{11}H$), 3.22 (3H, s, $C_{12}H$); **^{13}C NMR** (151 MHz, DMSO-d_6) δ ppm 161.0, 139.7, 138.9, 138.8, 134.4, 133.5, 129.1, 128.3, 126.3, 121.9, 120.3, 117.7, 115.8, 115.5, , 38.2, 29.2; **HPLC**: Retention time (system A): t_R= 7.59 min. Purity: >95%



4-Cyano-*N*-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-N-methylbenzenesulfonamide (185) Prepared by general procedure H from **102** (20 mg, 0.05 mmol). Purified by column chromatography (acetone:hexane ; 3:7) to give the title compound (13 mg, 0.03 mmol, 63%) as a colourless solid: **mp** 231– 233 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 8.09 (1H, d, *J* = 8.6 Hz, $C_{15}H$), 7.86 (1H, d, *J* = 9.6 Hz, C_4H), 7.82 (2H, d, *J* = 8.6 Hz, $C_{16}H$), 7.66 (1H, s, C_5H), 6.89 (1H, s, C_8H), 6.49 (1H, d, *J* = 9.3 Hz, C_3H), 3.62 (3H, s, $C_{12}H$), 3.50 (3H, s, $C_{11}H$), 3.19 (3H, s, $C_{13}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.3, 157.7, 142.7, 141.3, 138.6, 133.2, 131.3, 127.9, 123.0, 118.5, 117.8, 115.1, 113.5, 98.1, 55.6, 37.9, 29.3; **HRMS**: *m/z* ESI- [Found $(M+Cl)^-$ 418.0862, $C_{19}H_{17}N_3O_4S$ requires $(M+Cl)^-$ 418.0628]; **HPLC**: Retention time (system A): t_R= 9,39 min. Purity: >95%.

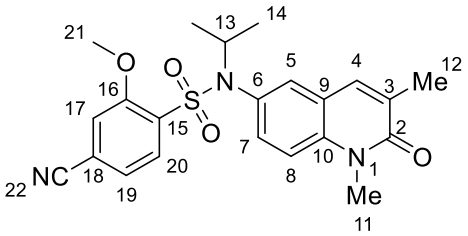


4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxy-N-methylbenzenesulfonamide (186) Prepared according to general procedure H from **NI-57** (50 mg, 0.13 mmol). Purified by column chromatography (acetone:hexane ; 1:9) to give the title compound (32 mg, 0.08 mmol, 62%) as a yellow solid: **mp** 227 – 229 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 7.82 (1H, d, *J* = 0.9 Hz), 7.75 - 7.72 (2H, m), 7.50-7.47 (2H, m), 7.45 - 7.41 (3H, m), 7.36 (3H, d, *J* = 2.2 Hz), 3.88 (3H, s), 3.61 (3H, s), 3.34 (3H, s), 2.11 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.5, 156.6, 137.5, 135.0, 134.5, 131.7, 130.4, 129.8, 127.6, 125.1, 124.1, 120.1, 117.5, 117.0, 116.9, 115.0, 56.8, 29.5, 17.3; **HPLC**: Retention time (system A): t_R= 9.98 min. Purity: >95%.

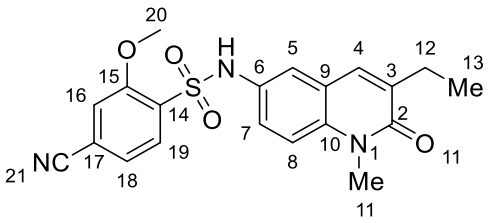


4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-*N*-ethyl-2-methoxybenzenesulfonamide (187) Prepared according to general procedure H from **NI-57** (50 mg, 0.13 mmol). Purified by column chromatography (acetone:hexane ; 1:9) to give the title

compound (44 mg, 0.11 mmol 82%) as a pale yellow solid: **mp** 187 – 188 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6) δ ppm 7.85 (1H, s, $C_{17}H$), 7.75 (1 H, s, C_4H), 7.66 (1H, d, J = 7.9 Hz, $C_{20}H$), 7.46 - 7.42 (3H, m, $C_8H/C_5H/C_{19}H$), 7.26 (1H, dd, J = 9.0, 2.4 Hz, C_7H), 4.00 (3H, s), 3.83 (2H, q, J = 6.9 Hz), 3.61 (3H, s), 2.11 (3H, s), 1.03 (3H, t, J = 7.1 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 162.3, 161.6, 156.5, 138.0, 135.0, 131.5, 131.2, 129.8, 129.8, 127.9, 124.0, 120.2, 117.6, 116.9, 116.7, 115.3, 56.9, 46.6, 29.5, 17.3, 14.6; **HRMS**: *m/z* ESI- [Found $(M+Cl)^-$ 446.0942 $C_{21}H_{21}N_3O_4S$ requires $(M+Cl)^-$ 446.0943]; **HPLC**: Retention time (system A): t_R= 10.69 min. Purity: >95%.

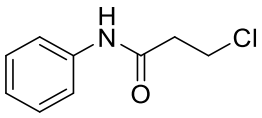


4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-*N*-isopropyl-2-methoxybenzenesulfonamide (188) Prepared according to general procedure H from **NI-57** (50 mg, 0.13 mmol). Purified by column chromatography (acetone:hexane ; 1:9) to give the title compound (27 mg, 0.06 mmol, 49%) as a pale yellow solid: **mp** 213 – 214 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 7.88 (1H, d, J = 0.9 Hz, $C_{17}H$), 7.77 (1H, s, C_4H), 7.66 (1H, d, J = 8.2 Hz, $C_{20}H$), 7.50 - 7.40 (2H, m, $C_{19}H/ C_{20}H$), 7.30 (1H, d, J = 2.2 Hz, C_5H), 7.08 (1H, dd, J = 8.8, 2.2 Hz, C_7H), 4.65 (1H, spt, J = 6.6 Hz, $C_{13}H$), 4.08 (3H, s, $C_{21}H$), 3.62 (3H, s, $C_{11}H$), 2.11 (3H, s, $C_{12}H$), 1.07 (6H, d, J = 6.6 Hz, $C_{14}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.6, 156.3, 138.6, 135.1, 133.0, 132.3, 131.2, 131.2, 129.7, 127.4, 124.0, 120.0, 117.6, 116.9, 116.6, 115.0, 56.8, 51.4, 29.5, 22.2, 17.3; **HRMS**: *m/z* ESI- [Found $(M+Cl)^-$ 460.1093 $C_{22}H_{23}N_3O_4S$ requires $(M+Cl)^-$ 460.1094]; **HPLC**: Retention time (system A): t_R= 11.14 min. Purity: >95%.

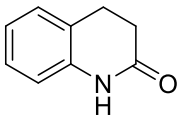


4-Cyano-*N*-(3-ethyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (191) Prepared according to general procedure E from **136** (30 mg, 0.15 mmol). Purified by column chromatography (acetone:hexane; 1:9-2:8) to give the title compound (23 mg, 0.06 mmol, 39%, or 12% over 2 steps) as a yellow solid: **mp** 198 – 199 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.37 (1H, s, *NH*), 7.92 (1H, d, J = 8.1 Hz, $C_{19}H$), 7.80 (1H, d, J = 1.3 Hz, $C_{16}H$), 7.71 (1H, s, C_4H), 7.54 (1H, dd, J = 8.1, 1.5 Hz, $C_{18}H$), 7.47 - 7.40 (2H, m, C_5H/ C_8H), 7.31 (1H, dd, J = 9.0, 2.4 Hz, C_7H), 4.04 (3H, s, $C_{20}H$), 3.61 (3H, s, $C_{11}H$), 1.20 (3H, t, J = 7.3 Hz, $C_{13}H$) *NB CH_2 overlaps with* DMSO; **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.7, 156.3, 156.3, 135.7, 135.2, 133.5, 131.1, 130.7, 124.1, 123.0, 120.3, 119.4, 117.5, 116.8, 116.7, 115.3, 57.0,

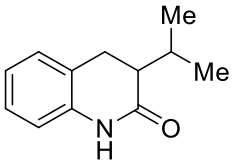
29.3, 23.7, 12.6.; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 396.1032 $C_{20}H_{19}N_3O_4S$ requires $(M-H)^-$ 396.1013]; **HPLC**: Retention time (system A): t_R= 9.85 min. Purity: >95%.



3-Chloro-*N*-phenylpropanamide (192) To a solution of aniline (9.3 g, 100 mmol, 1 eq.) in acetone (100 mL) and water (200 mL) was added K_2CO_3 (20.8 g, 150 mmol, 1.5 eq.). The suspension was cooled to 0 °C and 3-chloropropanoyl chloride (15.9 g, 125 mmol, 1.25 eq.) was added dropwise. The solution was maintained at 0 °C and stirred for 1 hour before being poured onto ice (~200 g). The precipitate was collected by filtration and dried under reduced pressure. To afford the desired compound, (18.1 g 97 mmol, 97%), as a white solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.54 (2H, d, *J* = 7.8 Hz), 7.46 - 7.30 (3H, m), 7.15 (1H, t, *J* = 7.6 Hz), 3.91 (2H, t, *J* = 6.3 Hz), 2.84 (2H, t, *J* = 6.4 Hz); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 167.8, 137.4, 129.1, 124.7, 120.1, 40.5, 39.9. The above data is in agreement with that previously reported in the literature. [131]

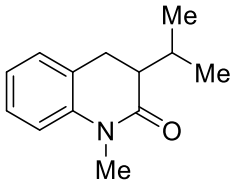


3,4-Dihydroquinolin-2(1*H*)-one (193) $AlCl_3$ (43 g, 328 mmol, 4 eq.) was added to **192** (15 g, 82 mmol, 1 eq.) under an argon atmosphere and the mixture was heated slowly to 120 °C and maintained at this temperature for 3 hours. The solution was allowed to cool to ~ 60 °C and then poured onto ice (~500 g) and stirred for 30 mins. The precipitate was filtered, washed with water and dried to provide the crude title product. Crystallisation of the crude material from MeOH gave the pure title compound (5.8 g, 39 mmol, 48%) as an off-white solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 8.66 (1H, br. s.), 7.23 - 7.13 (2 H, m), 7.01 (1H, td, *J* = 7.5, 1.0 Hz), 6.83 (1H, d, *J* = 7.8 Hz), 3.00 (2H, t, *J* = 7.6 Hz), 2.67 (2H, t, *J* = 7.3 Hz); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 171.6, 137.3, 128.0, 127.5, 123.7, 123.1, 115.7, 30.7, 25.3. The above data is in agreement with that previously reported in the literature. [132]

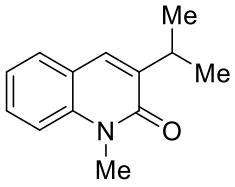


3-Isopropyl-3,4-dihydroquinolin-2(1*H*)-one (194) To a solution of **193** (1.5 g, 10 mmol, 1 eq.) in dry THF (50 mL) under an argon atmosphere at – 78 °C was added freshly prepared LDA (2.0M in THF, 25 mmol, 2.5 eq.) and the solution was stirred at – 78 °C for 1 hour. After this time 2-bromopropane (1.5 g, 12 mmol, 1.2 eq.) was added dropwise and stirred at this temperature for a further 30 minutes before being allowed to warm to RT. The solution was quenched with sat. NH_4Cl and extracted with EtOAc (3 × 50 mL). The organic fractions were pooled, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the residue by column

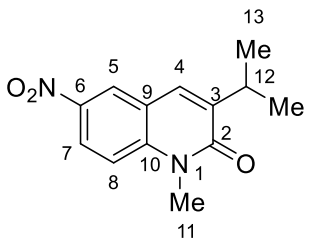
chromatography (ethyl acetate:hexane 1:9 – 3:7) gave the title compound (912 mg, 4.8 mmol, 48%) as a colorless solid: **mp** 152 – 153 °C (ethyl acetate-hexane); **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 8.63 (1H, br. s.), 7.23 - 7.13 (2H, m), 6.99 (1H, td, *J* = 7.5, 1.0 Hz), 6.80 (1H, dd, *J* = 8.1, 1.0 Hz), 3.04 – 2.84 (2H, m), 2.40 (1H, dt, *J* = 8.3, 6.2 Hz), 2.27 - 2.16 (1H, m), 1.07 (3H, d, *J* = 6.8 Hz), 1.00 (3H, d, *J* = 6.8 Hz); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 172.1, 140.3, 127.9, 127.3, 125.8, 122.6, 114.3, 47.0, 29.7, 27.2, 26.6, 20.9, 19.1.



3-Isopropyl-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (195) Prepared by general procedure B from **194** (280 mg, 1.48 mmol). Purified by column chromatography (hexane:EtOAc 1:9 – 2:8) gave the title compound (237 mg, 1.17 mmol, 79%) as a pale yellow oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.17 (1H, td, *J* = 7.8, 1.0 Hz), 7.09 (1H, d, *J* = 7.1 Hz), 6.93 (1H, t, *J* = 7.1 Hz), 6.88 (1H, d, *J* = 8.1 Hz), 3.29 (3H, s), 2.85 (1H, dd, *J*=15.7, 5.6 Hz), 2.73 (1H, dd, *J* = 16.1, 8.3 Hz), 2.27 (1H, dt, *J* = 8.2, 6.0 Hz), 2.08 - 2.02 (1 H, m), 0.92 (3H, d, *J* = 6.8 Hz), 0.85 (3H, d, *J* = 6.8 Hz); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 172.1, 140.3, 127.9, 127.3, 125.8, 122.6, 114.3, 47.0, 29.6, 27.2, 26.6, 20.8, 19.0.

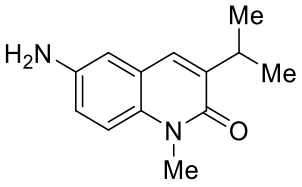


3-Isopropyl-1-methylquinolin-2(1*H*)-one (196) Prepared by general procedure I from **195** (520 mg, 2.56 mmol). 3 addition equivalents of DDQ added after 24 hours. Purified by column chromatography (hexane:EtOAc 1:9 – 2:8) gave the title compound (117 mg, 0.58 mmol, 23%) as a colourless oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 7.59 - 7.48 (3H, m), 7.35 (1H, d, *J* = 8.3 Hz), 7.24 (1H, td, *J* = 7.5, 1.0 Hz), 3.77 (3H, s), 3.33 (1H, spt, *J* = 7.1 Hz), 1.29 (6H, d, *J* = 6.8 Hz); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 162.2, 139.9, 138.8, 132.2, 129.3, 128.2, 121.9, 120.8, 113.7, 29.7, 28.2, 21.8.

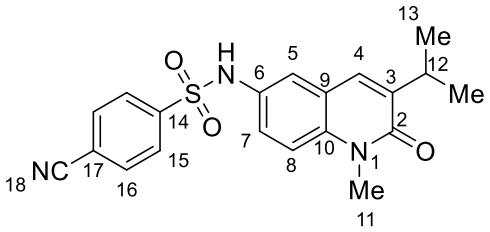


3-Isopropyl-1-methyl-6-nitroquinolin-2(1*H*)-one (197) To a solution of **196** (104 mg, 0.52 mmol) in AcOH (5 mL) at 0 °C was added KNO_3 (52 mg, 0.52 mmol, 1 eq.) in 1 portion and the resulting solution was stirred at 5 °C for 2 hours and then poured onto ice. The resulting suspension was filtered, washed with H_2O and dried to give the crude compound. Crystallisation

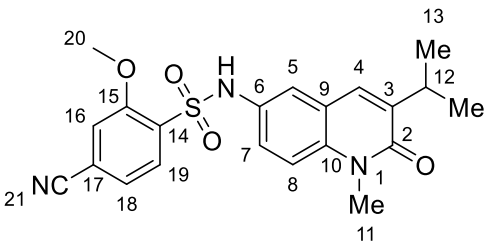
(EtOH) of the crude material provided the title compound (81 mg, 0.33 mmol, 63%) as pale yellow needles: **mp** 174 - 175 °C (ethanol); **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 8.49 (1H, d, *J* = 2.5 Hz, *C*$_5$*H*), 8.37 (1H, dd, *J* = 9.3, 2.5 Hz, *C*$_7$*H*), 7.60 (1H, s, *C*$_4$*H*), 7.43 (1H, d, *J* = 9.3 Hz, *C*$_8$*H*), 3.81 (3H, s, *C*$_{11}$*H*), 3.32 (1H, spt, *J* = 7.1 Hz, *C*$_{12}$*H*), 1.30 (6H, d, *J* = 7.1 Hz, *C*$_{13}$*H*); **^{13}C NMR** (101 MHz, $CDCl_3$): δ ppm 161.9, 142.7, 142.5, 142.0, 131.8, 124.0, 124.0, 120.2, 114.3, 30.3, 28.4, 21.6.



6-Amino-3-isopropyl-1-methylquinolin-2(1*H*)-one (198) Prepared according to general procedure D from **197** (75 mg, 0.30 mmol) to give the title compound as a yellow oil which was used without further purification.

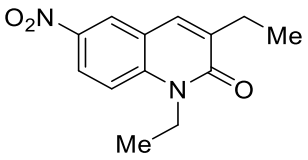


4-Cyano-*N*-(3-isopropyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (199) Prepared according to general procedure E from **198** (33 mg, 0.15 mmol). Purified by column chromatography (acetone:hexane; 2:8 – 4:6) to give the title compound (20 mg, 0.05 mmol, 34% over 2 steps) as a colourless solid: **mp** 209 – 210 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ ppm 10.54 (1H, s, *NH*), 8.04 (2H, d, *J* = 8.6 Hz, *C*$_{16}$*H*), 7.88 (2H, d, *J* = 8.3 Hz, *C*$_{15}$*H*), 7.67 (1H, s, *C*$_4$*H*), 7.44 (1H, d, *J* = 2.5 Hz, *C*$_5$*H*), 7.40 (1H, d, *J* = 9.1 Hz, *C*$_8$*H*), 7.22 (1H, dd, *J* = 9.0, 2.4 Hz, *C*$_7$*H*), 3.57 (3H, s, *C*$_{11}$*H*), 3.11 (1H, spt, *J* = 6.9 Hz, *C*$_{12}$*H*), 1.17 (6H, d, *J* = 7.1 Hz, *C*$_{13}$*H*); **^{13}C NMR** (126 MHz, DMSO-*d*$_6$): δ ppm 160.6, 143.4, 139.6, 135.9, 133.5, 131.8, 130.7, 127.4, 123.6, 120.5, 120.4, 117.5, 115.4, 115.3, 29.4, 27.8, 21.5; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 380.1063 $C_{20}H_{19}N_3O_3S$ requires $(M-H)^-$ 380.1069]; **HPLC**: Retention time (system A): t_R= 10.78 min. Purity: >95%.

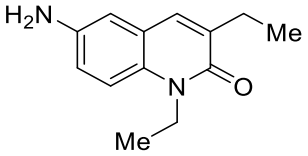


4-Cyano-*N*-(3-isopropyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (200) Prepared according to general procedure E from **198** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 4:6) to give the title compound (18 mg, 0.04 mmol, 29 % over 2 steps) as a colourless solid: **mp** 197 - 198 °C (acetone-

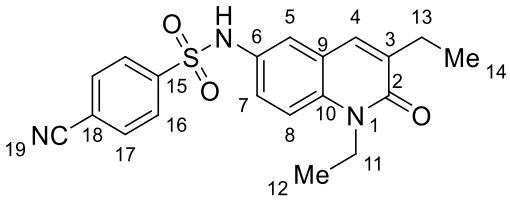
hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 10.27 (1H, s, *NH*), 7.86 (1H, d, *J* = 8.1 Hz, $C_{19}H$), 7.74 (1H, d, *J* = 1.3 Hz, $C_{16}H$), 7.63 (1H, s, C_4H), 7.47 (1H, dd, *J* = 8.1, 1.5 Hz, $C_{18}H$), 7.40 (1H, d, *J* = 2.3 Hz, C_5H), 7.37 (1H, d, *J* = 9.1 Hz, C_8H), 7.25 (1H, dd, *J* = 9.0, 2.4 Hz, C_7H), 3.99 (3H, s, $C_{20}H$), 3.56 (3H, s, $C_{11}H$), 3.10 (1H, spt, *J* = 6.8 Hz, $C_{12}H$), 1.16 (6H, d, *J* = 7.1 Hz, $C_{13}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.5, 156.3, 139.5, 135.6, 131.8, 131.0, 130.8, 124.1, 123.1, 120.2, 119.7, 117.5, 116.8, 116.7, 115.2, 57.0, 29.4, 27.9, 21.5; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 410.117 $C_{21}H_{21}N_3O_4S$ requires $(M-H)^-$ 410.1175]; **HPLC**: Retention time (system A): t_R= 10.60 min. Purity: >95%.



1,3-Diethyl-6-nitroquinolin-2(1*H*)-one (201) Prepared according to general procedure B from **135** (300 mg 1.38 mmol) to give the title compound (222 mg, 0.90 mmol, 65%) as a brown solid that was used without further purification.

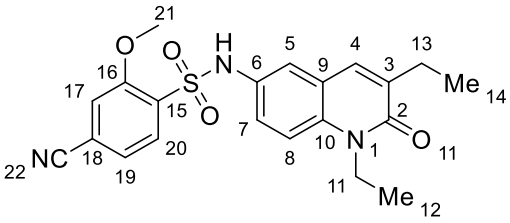


1,3-Diethyl-6-aminoquinolin-2(1*H*)-one (202) Prepared according to general procedure B from **201** (210 mg, 0.85 mmol) to give the title compound as a brown oil that was filtered through a silica plug (acetone:hexane ; 7:3) to give a yellow oil (81 mg, 38 mmol, 44%) that was used without further purification.

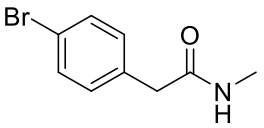


4-Cyano-*N*-(1,3-diethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (203) Prepared according to general procedure E from **202** (40 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 1:9 – 2:8) to give the title compound (37 mg, 0.09 mmol, 14% over 3 steps) as a pale yellow solid: **mp** 273 - 274 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.56 (1H, s), 8.04 (2H, d, *J* = 8.5 Hz, $C_{17}H$), 7.89 (2H, d, *J* = 8.5 Hz, $C_{116}H$), 7.68 (1H, s, C_4H), 7.46 (1H, d, *J* = 8.8 Hz, C_8H), 7.41 (1H, d, *J* = 2.5 Hz, C_5H), 7.23 (1H, dd, *J* = 9.1, 2.5 Hz, C_7H), 4.22 (2H, q, *J* = 6.9 Hz, $C_{11}H$), 1.21 - 1.10 (6H, m, $C_{12}H$/ $C_{14}H$) *NB* $C_{13}H$ *overlaps with* DMSO ; **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.5, 143.4, 135.3, 134.9, 133.6, 133.5, 130.7, 127.4, 123.6, 120.7, 120.3, 117.5,

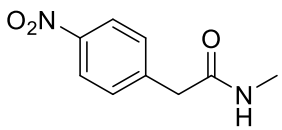
115.3, 115.2, 36.9, 23.5, 12.7, 12.5; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 380.1057 $C_{20}H_{19}N_3O_3S$ requires $(M-H)^-$ 380.1069]; **HPLC**: Retention time (system A): t_R= 10.36 min. Purity: >95%.



4-Cyano-*N*-(1,3-diethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (204) Prepared according to general procedure E from **202** (40 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane ; 1:9 – 2:8) to give the title compound (51 mg, 0.12 mmol, 20% over 3 steps) as a colorless solid: **mp** 210 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.30 (1H, s, *NH*), 7.88 (1H, d, *J* = 8.2 Hz, $C_{20}H$), 7.75 (1H, d, *J* = 0.9 Hz, $C_{17}H$), 7.64 (1H, s, C_4H), 7.48 (1H, dd, *J* = 8.2, 1.3 Hz, $C_{19}H$), 7.43 (1H, d, *J* = 8.8 Hz, C_8H), 7.36 (1H, d, *J* = 2.2 Hz, C_5H), 7.26 (1H, dd, *J* = 9.1, 2.5 Hz, C_7H), 4.20 (2H, q, *J* = 7.1 Hz, $C_{11}H$), 3.98 (3H, s, $C_{21}H$), 1.15 (6H, app. t, *J* = 7.4 Hz, $C_{12}H/C_{14}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 160.4, 156.3, 135.2, 134.6, 133.6, 131.0, 131.0, 130.9, 124.1, 123.1, 120.6, 119.5, 117.5, 116.8, 116.7, 115.0, 56.9, 36.9, 23.5, 12.7, 12.5; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 410.1185 $C_{21}H_{21}N_3O_4S$ requires $(M-H)^-$ 410.1253]; **HPLC**: Retention time (system A): t_R= 10.54 min. Purity: >95%.

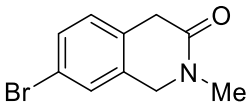


***N*-Methyl-2-(4-bromophenyl)acetamide (206a)** To a solution of 4-bromophenylacetic acid (5.0 g, 23.3 mmol, 1 eq.) and DMF (170 mg, 2.33 mmol, 0.1 eq.) in dry Toluene (12 mL) under an argon atmosphere was added thionyl chloride (3.32 g, 27.90 mmol, 1.2 eq.) dropwise. The solution was heated to 40 °C for 30 minutes and allowed to cool to RT. A solution of aqueous methylamine (40% by weight, 3.603 g, 10.0 mL, 116.25 mmol, 5 eq.) was cooled to 5 °C and the solution from the previous step was added dropwise with vigorous stirring at such a rate that the temperature of the solution did not exceed 25 °C. The resulting precipitate was filtered, washed with water (50 mL) and dried at 50 °C at 200 mbar to give the title compound (5.20 g, 22.8 mmol, 98%) as a colourless solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ 8.00 (1H, br. s.), 7.49 (2H, d, *J* = 8.20 Hz), 7.21 (2H, d, *J* = 8.20 Hz), 3.38 (2H, s), 2.57 (3H, d, *J* = 4.41 Hz). The above data is in agreement with that previously reported in the literature. [97]

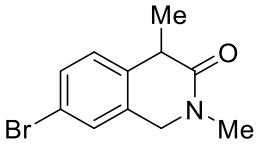


***N*-Methyl-2-(4-nitrophenyl)acetamide (206b)** Prepared as per Compound **206a** from 4-nitrophenylacetic acid (5.0 g, 27.6 mmol) to give the title compound (4.73 g, 24.9 mmol, 90%) as a

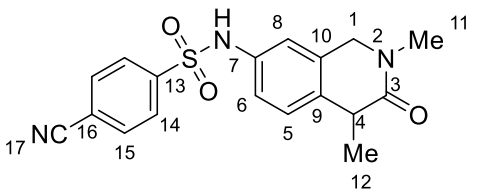
colourless solid: **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.17 (2H, d, *J* = 8.6 Hz), 8.06 (1H, s), 7.53 (2H, d, *J* = 8.6 Hz), 3.58 (2H, s), 2.60 (3 H, d, *J* = 4.5 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 169.3, 146.2, 144.5, 130.3, 123.3, 41.9, 25.6. The above data is in agreement with that previously reported in the literature. [97]



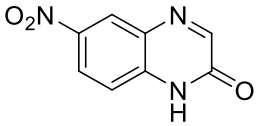
7-Bromo-2-methyl-1,4-dihydroisoquinolin-3(2H)-one (207) To a 3 necked 100 mL flask fitted with a reflux condenser, 50 mL dropwise addition funnel and a thermomemter was added Eaton's Reagent (25 mL) followed by **206a** (5 g, 21 mmol, 1 eq.) in portions. Paraformaldehyde (756 mg, 25.2 mmol, 1.2 eq.) was added and the solution was heated to 80 °C for 2 hours. After cooling to RT water (25 mL) was added dropwise and the resulting suspension was cooled to -5 °C. Sodium hydroxide (19 M, ~12 mL) was added and the suspension was filtered and the filtrate extracted with ethyl acetate (3 × 50 mL). The organic fractions were combined, concentrated under reduced pressure and the residue purified by column chromatography (EtOAc) to give the title compound (3.56 g, 14.8 mmol, 70%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.39 (1H, dd, *J* = 8.2, 1.9 Hz), 7.33 (1H, s), 7.04 (1H, d, *J* = 8.2 Hz), 4.47 (2H, s), 3.57 (2H, s), 3.11 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 168.2, 133.0, 131.3, 130.7, 129.0, 128.1, 120.2, 52.3, 36.4, 34.4. The above data is in agreement with that previously reported in the literature. [97]



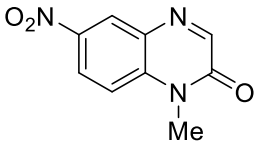
7-Bromo-2,4-dimethyl-1,4-dihydroisoquinolin-3(2*H*)-one (208): To a solution of diisopropylamine (632 mg, 6.25 mmol 1 eq.) in dry THF (12 mL) at -78 °C was added n-butyllithium (1.6 M in Hexane, 6.25 mmol, 1 eq.) dropwise. The solution was stirred for 25 minutes and **207** (1.5 g, 6.25 mmol, 1 eq.) in dry THF (6 mL) was added dropwise. Iodomethane (1.0 g, 7.5 mmol, 1.2 eq.) was added dropwise and stirred overnight. The solvent was removed under reduced pressure and the residue purified by column chromatography (6:4 – 10:0 ; EtOAc:hexane) to give the title compound (612 mg, 41 %) as a pale yellow oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.34 (1H, dd, *J* = 8.3, 1.8 Hz), 7.25 (1H, s), 7.01 (1H, d, *J* = 8.3 Hz), 4.37 (2H, dd, *J* = 35.1, 15.9 Hz), 3.43 (1H, q, *J* = 7.3 Hz), 3.03 (3H, s), 1.40 (3H, d, *J*=7.3 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 171.7, 137.0, 132.8, 130.8, 128.1, 128.1, 120.1, 51.6, 40.8, 34.7, 18.3.



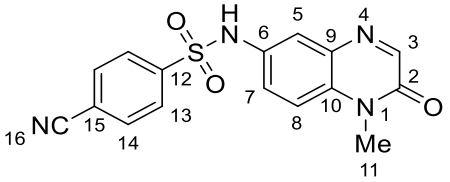
4-Cyano-*N*-(2,4-dimethyl-3-oxo-1,2,3,4-tetrahydroisoquinolin-7-yl)benzenesulfonamide (210) The required amine was prepared by general procedure J from **208** (77 mg, 0.30 mmol). Purified by column chromatography (EtOAc:hexane ; 9:1). Coupling carried out via general procedure E and purified by column chromatography (acetone:hexane ; 2:8) to give the title compound (17 mg, 0.05 mmol, 16% over 2 steps) as a pale yellow solid: **mp** 229 - 230 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.55 (1H, br. s *NH*), 8.05 (2H, d, *J* = 8.8 Hz, $C_{15}H$), 7.92 (2H, d, *J* = 8.6 Hz, $C_{14}H$), 7.13 (1H, d, *J* = 8.1 Hz, C_5H), 7.03 – 6.96 (2H, m, C_6H/C_8H), 4.47 (1H, d, *J* = 16.2 Hz, C_1H), 4.38 (1H, d, *J* = 16.2 Hz, C_8H), 3.38 (1H, q, *J* = 7.2 Hz, C_4H), 2.93 (3H, s, $C_{11}H$), 1.29 (3H, d, *J* = 7.3 Hz, $C_{12}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 170.7, 143.5, 135.0, 134.2, 133.5, 132.9, 127.4, 126.8, 119.6, 117.5, 117.4, 115.3, 50.8, 33.9, 17.0; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 354.0922 $C_{18}H_{17}N_3O_3$ requires $(M-H)^-$ 354.0912]; **HPLC**: Retention time (system A): t_R= 7.85 min. Purity: 89%.



6-Nitroquinoxalin-2(1*H*)-one (212) Prepared according to general procedure C from quinaxalin-2(1*H*)-one (2.50 g, 17.1 mmol) to give the title compound (3.00 g, 15.8 mmol, 92%) as a pale brown solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 12.92 (1H, br. s.), 8.55 (1H, d, *J* = 2.5 Hz), 8.39 (1H, dd, *J* = 9.1, 2.5 Hz), 8.33 (1H, s), 7.45 (1H, d, *J* = 9.1 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 154.7, 154.2, 142.5, 137.2, 130.8, 125.3, 124.3, 116.7. The above data is in agreement with that previously published in the literature. [133]

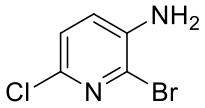


1-Methyl-6-nitroquinoxalin-2(1H)-one (213) Prepared according to general procedure B from **212** (1.0 g, 5.2 mmol). Crude product crystallised from EtOH to give the title compound (836 mg, 4.1 mmol, 78%) as orange needles: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.79 (1H, d, *J* = 2.5 Hz), 8.48 (1H, dd, *J* = 9.1, 2.5 Hz), 8.44 (1H, s), 7.48 (1H, d, *J* = 9.1 Hz), 3.78 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 154.5, 152.5, 143.4, 137.9, 132.5, 126.3, 125.6, 114.5, 29.3. The above data is in agreement with that previously published in the literature. [134]

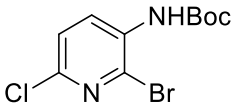


4-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinoxalin-6-yl)benzenesulfonamide (215) Amine prepared by general procedure D from **213** (100 mg, 0.48 mmol). The crude amine was then coupled *via* general procedure E. Purified by column chromatography (acetone:hexane ; 4:6 – 6:4) to give the title compound (14 mg, 0.04 mmol, 8% over 2 steps) as a yellow solid: **mp** 247 °C

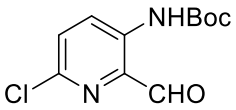
(decomposed); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.77 (1H, br. s. *NH*), 8.21 (1H, s, *C*$_3$*H*), 8.06 (2H, d, *J* = 8.8 Hz, *C*$_{14}$*H*), 7.92 (2H, d, *J* = 8.5 Hz, *C*$_{13}$*H*), 7.52 (1H, d, *J* = 8.8 Hz, *C*$_8$*H*), 7.49 (1H, d, *J* = 2.2 Hz, *C*$_5$*H*), 7.39 (1H, dd, *J* = 9.0, 2.4 Hz, *C*$_7$*H*), 3.52 (3H, s, *C*$_{11}$*H*); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 154.0, 151.2, 143.2, 133.6, 132.7, 132.4, 130.5, 127.4, 124.6, 120.8, 117.5, 116.0, 115.5, 28.6; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 339.0562, $C_{16}H_{12}N_4O_3S$ requires $(M-H)^-$ 339.0552]; **HPLC**: Retention time (system A): t_R= 6.99 min. Purity: >95%.



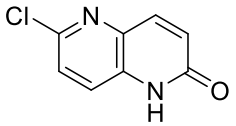
2-Bromo-6-chloropyridin-3-amine (217) Prepared according to the procedure of Grongsaard *et al* from 6-chloropyridin-3-amine (5.0 g, 45.0 mmol) to give the title compound (7.65 g, 36.9 mmol, 82%) as a brown solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.11 (1H, d, *J* = 8.2 Hz), 7.01 (1H, d, *J* = 8.2 Hz), 4.17 (2H, br. s.); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 140.5, 137.7, 126.7, 124.4, 123.9. The above data is in agreement with that previously published in the literature. [98]



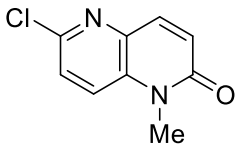
***Tert*-butyl (2-bromo-6-chloropyridin-3-yl)carbamate (218)** Prepared according to the procedure of Grongsaard *et al* from **217** (3.25 g, 15.7 mmol) to give the title compound (3.32 g, 10.8 mmol, 69%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.39 (1H, d, *J* = 8.5 Hz), 7.19 (1H, d, *J* = 8.8 Hz), 6.92 (1H, br. s.), 1.47 (9H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 151.9, 142.5, 133.5, 129.7, 129.2, 123.8, 82.2, 28.2. The above data is in agreement with that previously published in the literature. [98]



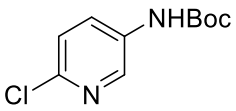
***Tert*-butyl (4-bromo-2-formylpyridin-3-yl)carbamate (219)** Prepared according to the procedure of Grongsaard *et al* from **218** (1.5 g, 4.88 mmol) to give the title compound (603 mg, 2.00 mmol, 41%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 10.11 (1H, br. s.), 9.92 (1H, s), 8.81 (1H, d, *J* = 9.1 Hz), 7.40 (1H, d, *J* = 9.1 Hz), 1.47 (9H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 195.7, 152.5, 143.3, 138.3, 135.8, 129.9, 129.8, 82.2, 27.2. The above data is in agreement with that previously published in the literature. [98]



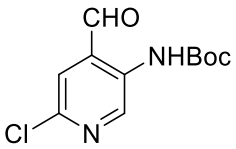
6-Chloro-1,5-naphthyridin-2(1*H*)-one (220) Prepared according to general procedure M from **219** (400 mg, 1.56 mmol) to give the title compound (106 mg, 0.59 mmol, 38%) as yellow needles: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 12.39 (1H, br. s.), 8.64 (1H, d, *J* = 8.9 Hz), 8.31 (1H, d, *J* = 9.6 Hz), 7.92 (1H, d, *J* = 9.0 Hz), 7.01 (1H, d, *J* = 9.6 Hz).



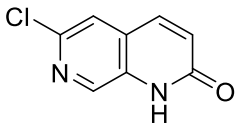
6-Chloro-1-methyl-1,5-naphthyridin-2(1*H*)-one (221) Prepared according to general procedure B from **x** (125 mg, 0.69 mmol). Purified by column chromatography (acetone:hexane ; 2:8) to give the title compound (87 mg, 0.45 mmol, 65%) as a pale yellow solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.47 (1H, d, *J* = 8.8 Hz), 8.21 (1H, d, *J* = 9.5 Hz), 7.85 (1H, d, *J* = 9.0 Hz), 7.11 (1H, d, *J* = 9.5 Hz), 3.74 (3H, s).



***Tert*-butyl (6-chloropyridin-3-yl)carbamate (222)** Prepared according to the procedure of Rewcastle *et al* from 6-chloropyriding-3-amine (3.5 g, 27.3 mmol) to give the title compound (5.49 g, 24.0 mmol, 88%) as an orange solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.26 (1H, d, *J* = 2.8 Hz), 7.99 (1H, br. s.), 7.28 (1H, d, *J* = 8.5 Hz), 6.61 (1H, br. s.), 1.54 (9H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 152.3, 144.8, 139.4, 134.3, 128.5, 124.2, 81.6, 28.2. The above data is in agreement with that previously published in the literature. [135]

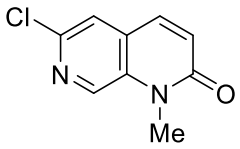


***Tert*-butyl (6-chloro-4-formylpyridin-3-yl)carbamate (223)** Prepared according to the procedure of Leivers *et al* from **222** (4.0 g, 17.5 mmol) to give the title compound (1.66 g, 6.47 mmol, 37%) as a pale yellow solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 9.97 (1H, s), 9.79 (1H, br. s.), 9.64 (1H, s), 7.57 (1H, s), 1.57 (9H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 193.4, 152.0, 143.8, 142.3, 134.7, 127.2, 126.9, 82.1, 28.2. The above data is in agreement with that previously published in the literature. [136]

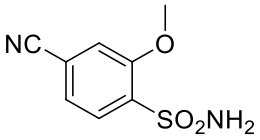


6-Chloro-1,7-naphthyridin-2(1*H*)-one (224) Prepared according to general procedure M from **223** (750 mg, 2.93 mmol) to give the title compound (265 mg, 1.46 mmol, 50%) as yellow needles: **mp** 109 -111 °C (DMF-water); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 12.10 (1H, br. s.), 8.44 (1H, s),

7.92 (1H, d, *J* = 9.8 Hz), 7.83 (1H, s), 6.83 (1H, d, *J* = 9.5 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.2, 141.5, 137.6, 137.4, 133.9, 128.4, 126.7, 120.5.

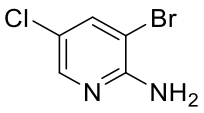


6-Chloro-1-methyl-1,7-naphthyridin-2(1*H*)-one (225) Prepared according to general procedure B from **224** (250 mg, 1.38 mmol). Purified by column chromatography (acetone:hexane ; 2:8) to give the title compound (215 mg, 1.11 mmol, 80%) as a pale yellow solid: **mp** 217 – 218 °C (acetone-hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.59 (1H, s), 7.61 (1H, d, *J* = 9.5 Hz), 7.49 (1H, s), 6.96 (1H, d, *J* = 9.5 Hz), 3.78 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.0, 143.9, 136.3, 135.9, 134.6, 128.3, 128.1, 120.9, 29.2.



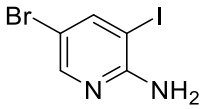
4-Cyano-2-methoxybenzenesulfonamide (227) 4-mercapto-3-methoxybenzonitrile (2.0 g, 12.1 mmol, 1 eq.) and $ZrCl_4$ (2.8 g, 12.1 mmol, 1eq.) were suspended in dry MeCN (60 mL) under an argon atmosphere and then stirred until homogenous. The resulting solution was cooled to 0 °C and H_2O_2 (30% w/w in H_2O, 3.7 mL, 36.4 mmol, 3 eq.) was added dropwise, stirred for 2 hours at 0 °C and then allowed to warm to RT. The suspension was quenched with H_2O (20 mL) and extracted with EtOAc (3 × 50 mL). The organic fractions were combined, dried over *anhydrous* sodium sulfate, filtered and concentrated under reduced pressure to give crude 4-cyano-2-methoxybenzenesulfonyl chloride which was used without purification in the next step.

The crude 4-cyano-2-methoxybenzenesulfonyl chloride was dissolved in conc. NH_4OH (10 mL) and stirred overnight. The solvent was concentrated under reduced pressure, the residue dissolved in EtOH (50 mL) and the resulting suspension filtered. The filtrate was concentrated under reduced pressure and the residue crystallised (EtOH:H_2O ; 2:1) to give the title compound (582 mg, 2.72 mmol, 23% over 2 steps) as pale yellow needles: **mp** 221 - 223 °C (ethanol-water); **^{1}H NMR** (500 MHz, DMSO-d_6): δ 7.88 (1H, d, *J* = 8.2 Hz), 7.75 (1H, d, *J* = 1.3 Hz), 7.55 (1H, dd, *J* = 7.9, 1.3 Hz), 7.39 (2H, s), 3.97 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 156.0, 135.4, 128.5, 124.0, 117.8, 116.4, 115.7, 56.8.

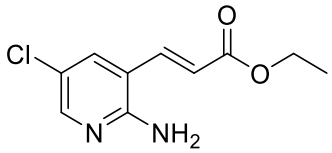


3-Bromo-5-chloropyridin-2-amine (229a) Prepared according to the procedure of Murtiashaw *et al* from 5-chloropyridine-2-amine (3.0 g, 23.4 mmol) to give the title compound (3.0 g, 14.5 mmol, 62%) as a brown solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 8.00 (1H, d, *J* = 2.0 Hz), 7.68 (1H, d,

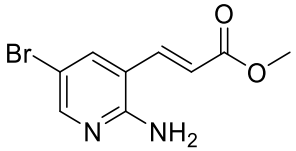
J = 2.3 Hz), 4.94 (2H, br. s.); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 153.6, 145.4, 139.6, 119.6, 102.8. The above data is in agreement with that previously published in the literature. [137]



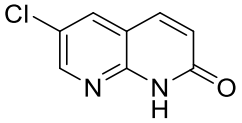
5-Bromo-3-iodopyridin-2-amine (229b) Prepared according to the procedure of Hibi *et al* from 4-bromo-2-aminopyridine (5.0 g, 28.9 mmol) to give the title compound (4.04 g, 13.58 mmol, 47%) as an orange solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.08 (1H, d, *J* = 2.2 Hz), 7.97 (1H, d, *J* = 1.9 Hz), 5.03 (2H, br. s.); **^{13}C NMR** (126 MHz, $CDCl_3$) δ ppm 156.4, 148.5, 148.2, 107.3, 77.6. The above data is in agreement with that previously published in the literature. [138]



Ethyl (*E*)-3-(2-amino-5-chloropyridin-3-yl)acrylate (230a) Prepared according to general procedure P from **229a** (2.2 g, 10.6 mmol). Purification by column chromatography (EtOAc:hexane ; 1:1) gave the title compound (1.41 g, 6.27 mmol, 59%) as a bright yellow solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.08 (1H, d, *J* = 2.2 Hz), 7.61 (1H, s), 7.60 (1H, d, *J* = 15.6 Hz), 6.40 (1H, d, *J* = 15.8 Hz), 4.79 (2H, br. s.), 4.30 (2H, q, *J* = 7.3 Hz), 1.36 (3H, t, *J* = 7.1 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 166.3, 154.8, 148.2, 137.5, 135.3, 121.7, 121.4, 115.5, 60.5, 14.2. The above data is in agreement with that previously published in the literature. [139]

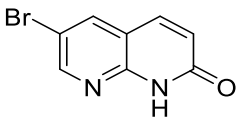


Methyl (*E*)-3-(2-amino-5-bromopyridin-3-yl)acrylate (230b) Prepared according to general procedure P from **229b** (3.0 g, 10.1 mmol). Purified by column chromatography (EtOAc:hexane ; 1:9 – 3:7) to give the title compound (1.68 g, 6.5 mmol, 65 %) as a bright yellow solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.07 (1H, d, *J* = 2.2 Hz), 7.64 (1H, d, *J* = 2.2 Hz), 7.50 (1H, d, *J* = 15.8 Hz), 6.30 (1H, d, *J* = 15.8 Hz), 4.68 (2H, br. s.), 3.75 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 166.6, 155.0, 150.5, 137.9, 137.7, 120.8, 116.0, 108.8, 52.0.

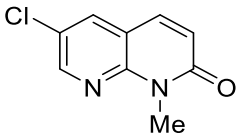


6-Chloro-1,8-naphthyridin-2(1*H*)-one (231a) Prepared by general procedure Q from **230a** (1.0 g, 4.72 mmol) to give the title compound (747 mg, 4.15 mmol, 88%) as a colourless solid**: mp** >300 °C (methanol-water); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.51 (1H, d, *J* = 2.5 Hz), 8.22 (1H, s), 7.83

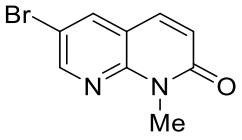
(1H, d, 9.5 Hz), 6.59 (1H, d, 9.5 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 169.8, 165.5,156.1, 148.3, 137.4, 134.8, 124.2, 116.9, 106.8.



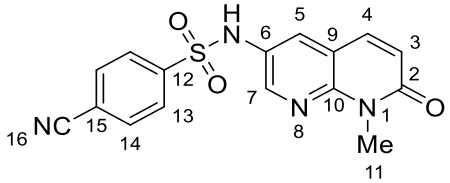
6-Bromo-1,8-naphthyridin-2(1*H*)-one (231b) Prepared by general procedure Q from **230b** (1.0 g, 3.89 mmol) to give the title compound (665 mg, 2.96 mmol, 76%) as a colourless solid: **mp** >300 °C (methanol-water); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 8.39 (1 H, d, *J*=2.8 Hz), 8.01 (1 H, d, *J*=2.5 Hz), 7.55 (1 H, d, *J*=9.1 Hz), 6.38 (1 H, d, *J*=8.8 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 170.8, 165.7, 156.5, 149.5, 136.2, 135.0, 123.3, 116.6, 106.7.



6-Chloro-1-methyl-1,8-naphthyridin-2(1*H*)-one (232a) Prepared by general procedure B from **231a** (250 mg, 1.39 mmol). Purified by column chromatography (EtOAc:hexane ; 3:7) to give the title compound (191 mg, 0.98 mmol, 71%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.39 (1H, d, *J* = 2.0 Hz), 7.98 (1H, d, *J* = 2.0 Hz), 7.33 (1H, d, *J* = 9.5 Hz), 6.59 (1H, d, *J* = 9.8 Hz), 3.89 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 161.9, 152.5, 148.0, 135.2, 132.9, 124.9, 116.8, 114.0, 28.2.

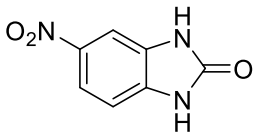


6-Bromo-1-methyl-1,8-naphthyridin-2(1*H*)-one (232b) Prepared by general procedure B from **231b** (300 mg, 1.33 mmol). Purified by column chromatography (EtOAc:hexane ; 3:7) to give the title compound (258 mg, 1.08 mmol, 81%) as a colourless solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 8.64 (1H, d, *J* = 2.2 Hz), 8.01 (1H, d, *J* = 2.2 Hz), 7.59 (1H, d, *J* = 9.5 Hz), 6.82 (1H, d, *J* = 9.8 Hz), 3.82 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.7, 150.5, 148.7, 137.9, 135.8, 124.3, 116.9, 113.1, 28.4.

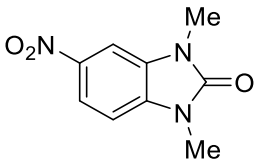


4-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinoxalin-6-yl)benzenesulfonamide (234) Amine prepared by general procedure J from **232b** (100 mg, 0.42 mmol). The crude amine was then

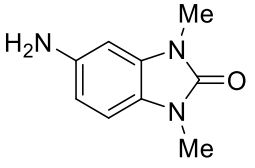
coupled *via* general procedure E. Purified by column chromatography (acetone:hexane ; 4:6 – 6:4) to give the title compound (18 mg, 0.05 mmol, 13% over 2 steps) as a yellow solid**: mp** 283 – 284 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.80 (1H, br. s. *NH*), 8.28 (1H, d, *J* = 2.5 Hz, C_7H), 8.05 (2H, d, *J* = 8.5 Hz, $C_{14}H$), 7.97 (1H, d, *J* = 9.8 Hz, C_4H), 7.92 (1H, d, *J* = 2.5 Hz, C_5H), 7.90 (2H, d, *J* = 8.5 Hz, $C_{13}H$), 6.71 (1H, d, *J* = 9.8 Hz, C_3H), 3.61 (3H, s, $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.8, 147.2, 144.2, 142.9, 137.3, 133.6, 130.0, 128.1, 127.4, 123.0, 117.5, 115.5, 115.2, 27.8; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 339.0553, $C_{16}H_{12}N_4O_3S$ requires $(M-H)^-$ 339.0552]; **HPLC**: Retention time (system A): t_R= 6.21 min. Purity: >95%.



5-Nitro-1,3-dihydro-*2H*-benzo[*d*]imidazol-2-one (236) Prepared according to the procedure of Belvagi *et al* from 1,2-diamino-4-nitrobenzene (2.50 g, 16.3 mmol) to give the title compound (2.69 g, 15.0 mmol, 90%) as an orange solid that was used without further purification. Analytical sample prepared by crystallisation (EtOH:H_2O ; 1:1). 380 mg of crude gave 300 mg of pure compound: **^{1}H NMR** (500 MHz, DMSO-d_6): δ 11.41 (1H, br. s.), 11.18 (1H, br. s.), 7.95 (1H, dd, *J* = 8.7, 2.4 Hz), 7.71 (1H, d, *J* = 2.2 Hz), 7.11 (1H, d, *J* = 8.5 Hz); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 155.3, 141.2, 135.8, 129.5, 117.8, 108.0, 103.6. The above data is in agreement with that previously reported in the literature.[140]

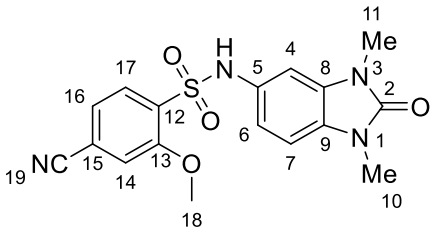


1,3-Dimethyl-5-nitro-1,3-dihydro-*2H*-benzo[*d*]imidazol-2-one (237) Prepared according to general procedure B from **236** (1.50 g, 8.30 mmol). Crude residue crystallised from EtOH to give the title compound (798 mg, 3.85 mmol, 46%) as yellow needles: **^{1}H NMR** (500 MHz, $CDCl_3$): δ 8.14 (1H, dd, *J* = 8.7, 2.0 Hz), 7.91 (1H, d, *J* = 2.2 Hz), 7.05 (1H, d, *J* = 8.8 Hz), 3.52 (3H, s), 3.51 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 154.7, 142.6, 134.9, 129.7, 118.5, 106.5, 103.2 , 27.6, 27.6.The above data is in agreement with that previously reported in the literature. [141]

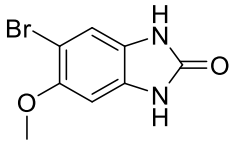


5-Amino-1,3-dimethyl-1,3-dihydro-*2H*-benzo[*d*]imidazol-2-one (238) 237 (500 mg, 2.41 mmol, 1 eq.) and palladium on carbon (5% by weight, 514 mg, 0.24 mmol, 0.1 eq.) were placed in a sealed rbf and backfilled with nitrogen 2 times and then EtOH (40 mL) was added. The flask was fitted with a balloon of hydrogen and purged and then backfilled 5 times. The resulting suspension

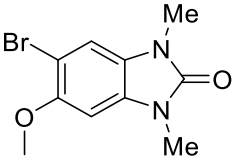
was stirred at RT overnight, purged with nitrogen for 20 mins and then filtered through a pad of Celite ® eluting with EtOH. The filtrate was concentrated under reduced pressure to give the title compound (350 mg, 1.98 mmol, 82%) as a white solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ 6.76 (1H, d, *J* = 8.2 Hz), 6.47 (1H, dd, *J* = 8.2, 1.9 Hz), 6.39 (1H, d, *J* = 1.9 Hz), 3.61 (2H, br. s.), 3.38 (3H, s), 3.37 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 154.8, 141.5, 131.1, 123.0, 108.2, 107.9, 95.6, 27.1, 27.1. The above data is in agreement with that previously reported in the literature. [141]



4-Cyano-*N*-(1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzo[d]imidazol-5-yl)-2-methoxybenzenesulfonamide (239) Prepared by general procedure E from **238** (20 mg, 0.11 mg). Purified by column chromatography (acetone:hexane ; 4:6) to give the title compound (23 mg, 0.06 mmol, 55%) as a pale yellow solid: **mp** 251 – 252 °C (acetone-hexane); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.04 (1H, s, *NH*), 7.80 (1H, d, *J* = 7.8 Hz, $C_{17}H$), 7.73 (1H, d, *J* = 1.3 Hz, $C_{14}H$), 7.45 (1H, dd, *J* = 8.0, 1.4 Hz, $C_{16}H$), 6.95 (1H, d, *J* = 8.3 Hz, C_7H), 6.85 (1H, d, *J* = 1.8 Hz, C_4H), 6.71 (1H, dd, *J* = 8.3, 1.8 Hz, C_6H), 4.01 (3H, s, $C_{18}H$), 3.23 (3H, s, $C_{10}H$ *or* $C_{11}H$), 3.23 (3H, s, $C_{10}H$ *or* $C_{11}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 156.2, 153.7, 131.1, 130.8, 130.5, 129.8, 127.1, 124.0, 117.5, 116.7, 116.5, 114.7, 107.7, 102.1, 56.9, 26.9, 26.8; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 371.0821 $C_{17}H_{16}N_4O_4S$ requires $(M-H)^-$ 371.0814]; **HPLC**: Retention time (system A): t_R= 7.64 min. Purity: >95%.

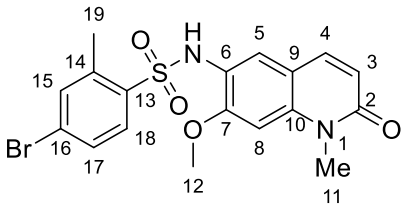


5-Bromo-6-methoxy-1,3-dihydro-*2H*-benzo[*d*]imidazol-2-one (241) : 5-methoxy-1,3-dihydro-*2H*-benzo[*d*]imidazol-2-one (400 mg, 2.44 mmol, 1 eq.) was dissolved in DMF (10 mL) and NBS (434 mg, 2.44 mmol, 1 eq.) was added in one portion. The solution was stirred overnight and poured onto crushed ice (~ 100 g). The resulting precipitate was filtered and dried to give the title compound (498 mg, 2.05 mmol, 84%) as a pale brown solid: **mp** 276 - 278 °C (decomposed);**^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.74 (1H, s), 10.52 (1H, s), 7.05 (1H, s), 6.71 (1H , s), 3.79 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 155.2, 150.2, 130.1, 124.4, 112.1, 101.3, 95.0, 56.6.

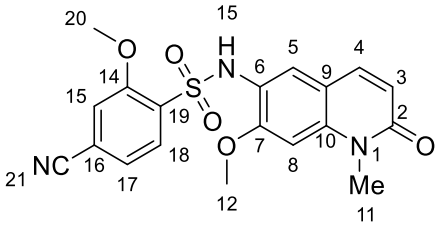


5-Bromo-6-methoxy-1,3-dimethyl-1,3-dihydro-*2H*-benzo[*d*]imidazol-2-one (242) Prepared according to general procedure B from **241** (300 mg, 1.23 mmol, 1 eq.). Product precipitated and

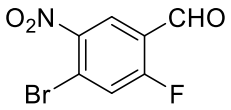
was filtered, washed with H_2O and dried to give the title compound (327 mg, 1.20 mmol, 98%) as a brown solid: **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 7.40 (1H, s), 7.07 (1H, s), 3.87 (3H, s), 3.29 (3H, m) *NB NCH_3 peak overlaps with DMSO H_2O peak;* **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 153.9, 150.7, 129.9, 124.2, 111.6, 101.8, 94.6, 56.9, 27.1, 27.1.



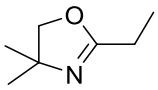
4-Bromo-N-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methylbenzenesulfonamide (243) Prepared by general procedure E from a solution of **93** in NMP (78 mg, 0.38 mmol). Purified by column chromatography (acetone:hexane ; 2:8 – 4:6) to give the title compound (37 mg, 0.08 mmol, 22% over 2 steps) as a brown solid: **mp** 207 - 208 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.78 (1H, s, *NH*), 7.84 (1H, d, *J* = 9.5 Hz, C_4H), 7.68 (1H, s, $C_{15}H$), 7.59 (1H, s, C_5H), 7.47 (1H, d, *J* = 8.5 Hz, $C_{18}H$), 7.44 (1H, d, *J* = 8.5 Hz, $C_{17}H$), 6.82 (1H, s, C_8H), 6.45 (1H, d, *J* = 9.5 Hz, C_3H), 3.59 (3H, s, $C_{12}H$), 3.57 (3H, s, $C_{11}H$), 2.66 (3H, s, $C_{19}H$); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.2, 155.9, 139.9, 139.9, 138.7, 137.8, 134.5, 130.8, 128.6, 127.5, 126.1, 119.5, 118.3, 113.3, 97.5, 55.7, 29.2, 19.5; **HRMS**: *m/z* ESI- [Found $(M+H)^+$ 435.0013, $C_{18}H_{17}N_2O_4SBr$ requires $(M+H)^+$ 435.0014]; **HPLC**: Retention time (system A): t_R= 10.40 min. Purity: >95%.



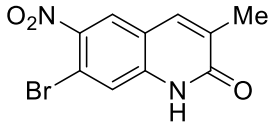
4-Cyano-2-methoxy-N-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (244) Prepared by general procedure E from a solution of **93** in NMP (78 mg, 0.38 mmol). Purified by column chromatography (acetone:hexane ; 1:9 – 1:1) to give the title compound (24 mg, 0.06 mmol, 16% over 2 steps) as a yellow solid: **mp** 211 - 213 °C (acetone-hexane); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 9.43 (1H, s, *NH*), 7.83 (1H, d, *J* = 9.5 Hz, C_4H), 7.78 (1H, d, *J* = 0.9 Hz, $C_{15}H$), 7.70 (1H, d, *J* = 7.9 Hz, $C_{18}H$), 7.58 (1H, s, C_5H), 7.43 (1H, dd, *J* = 8.2, 1.3 Hz, $C_{17}H$), 6.84 (1H, s, C_8H), 6.44 (1H, d, *J* = 9.5 Hz, C_3H), 3.98 (3H, s, $C_{12}H$), 3.65 (3H, s, $C_{12}H$), 3.58 (3H, s, C_1H); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 161.2, 156.8, 15, 5.6, 139.7, 138.7, 132.4, 130.0, 127.1, 123.6, 119.9, 118.3, 117.7, 116.3, 116.2, 113.3, 97.4, 56.9, 55.9, 29.2; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 398.0803, $C_{19}H_{17}N_3O_5S$ requires $(M-H)^-$ 398.0811]; **HPLC**: Retention time (system A): t_R= 8.22 min. Purity: >95%.



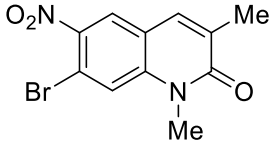
4-Bromo-2-fluoro-5-nitrobenzaldehyde (246) Prepared according to the procedure of Kusakabe *et al* from 2-fluoro-4-bromobenzaldeyde (5.00 g, 24.6 mmol) to give the title compound (5.44 g, 21.9 mmol, 89%) as a pale yellow solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 10.33 (1H, s), 8.43 (1H, d, *J* = 6.3 Hz), 7.69 (1H, d, *J* = 9.1 Hz); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 183.9 (d, *J* = 5.5 Hz), 164.1 (d, *J* = 269.4 Hz), 126.1 (d, *J* = 3.7 Hz), 124.1, 123.9, 123.5 (d, *J* = 10.1 Hz), 122.6 (d, *J* = 11.0 Hz). The above data is in agreement with that previously reported in the literature. [101]



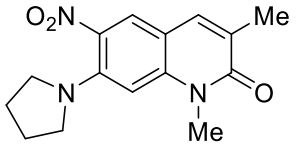
2-Ethyl-4,4-dimethyl-4,5-dihydrooxazole (247) Prepared as per compound **133** from propionic acid (5.0 g, 67.6 mmol) to give the title compound (4.72 g, 37.1 mmol, 55%) as a colourless oil: **^{1}H NMR** (400 MHz, $CDCl_3$): δ ppm 3.90 (2H, s), 2.28 (2H, q, *J* = 7.5 Hz), 1.28 (6H, s), 1.16 (3H, t, *J* = 7.5 Hz) .



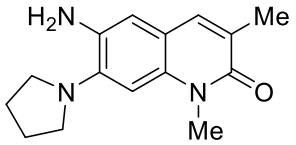
7-Bromo-3-methyl-6-nitroquinolin-2(1*H*)-one (248) Prepared according to general procedure F from 2-fluoro-4-nitro-5-bromo-benzaldehyde (4.20 g, 16.8 mmol, 1 eq.) and **247** (2.13 g, 16.8 mmol, 1 eq.) to give the title compound (3.92 g, 13.8 mmol, 82%) as a black solid that was used without further purification: Relevant **^{1}H NMR** (500 MHz, DMSO-d_6) δ ppm 12.22 (1H, br. s.), 8.46 (1H, s), 7.90 (1H, s), 7.63 (1H, s), 2.10 (3H, d, *J*=0.9 Hz).



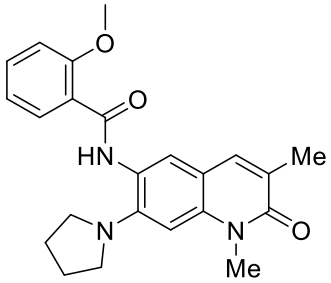
7-Bromo-1,3-methyl-6-nitroquinolin-2(1*H*)-one (249) Prepared by general procedure B from **248** (1.2 g, 4.2 mmol). Product precipitated on addition of H_2O and was filtered, washed with H_2O and dried to give the title compound (983 mg, 3.30 mmol, 78%) as a brown solid that was used without further purification: Relevant **^{1}H NMR** (500 MHz, DMSO-d_6) δ ppm 8.57 (1H, s), 8.04 (1H, s), 8.00 (1H, s), 3.77 (3H, s), 2.23 (3H, s).



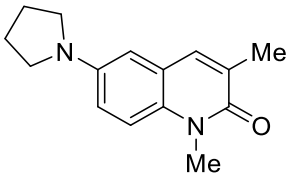
1,3-Methyl-6-nitro-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (250) - 249 (100 mg, 0.35 mmol, 1 eq.), CuI (7 mg, 0.04 mmol, 0.1 eq.) and pyrrolidine (125 mg, 1.75 mmol, 5 eq.) were dissolved in dry DMF and heated at 80 °C for 3 hours. After cooling to RT the solution was poured onto ice (~50 g) stirred for 10 mins and filtered to give the title compound (62 mg, 0.22 mmol, 65%) as a brown solid: **mp** 184 °C (decomposed); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.96 (1H, s), 7.43 (1H, s), 6.51 (1H, s), 3.70 (3H, s), 3.41 - 3.21 (4H, m), 2.23 (3H, s), 2.11 – 1.98 (4H, m) ; **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 163.5, 143.1, 142.7, 135.0, 133.6, 126.8, 126.7, 110.7, 98.3, 50.9, 29.8, 25.8, 17.5.



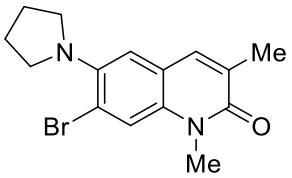
6-Amino-1-methyl-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (251) Prepared by general procedure D from **250** (40 mg, 0.11 mmol). Compound precipitated on basification to pH 11 and was filtered, washed with H_2O and dried to give the title compound (24 mg, 0.1 mmol, 91%) as a dark red solid: **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 7.40 (1H, s), 6.84 (1H, s), 6.81 (1H, s), 3.85 - 3.70 (5H, m), 3.31 – 3.14 (4H, m), 2.24 (3H, s), 2.07 – 1.98 (4H, m) ; **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 162.7, 135.7, 134.7, 133.4, 131.2, 127.2, 116.3, 112.7, 102.8, 50.8, 29.6, 24.3, 17.8.



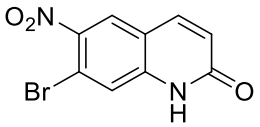
2-Methoxy-*N*-(1-methyl-2-oxo-7-(pyrrolidin-1-yl)-1,2-dihydroquinolin-6-yl)benzamide (252) Prepared by general procedure E from **251** (20 mg, 0.08 mmol). Purified by column chromatography (acetone:hexane 2:8) to give the title compound (12 mg, 0.03 mmol, 36%) as an orange solid: **mp** 193 - 196 °C (acetone-hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ ppm 10.12 (1H, s), 8.36 (1H, s), 8.30 (1H, dd, *J* = 7.7, 1.7 Hz), 7.48 - 7.42 (2H, m), 7.10 (1H, t, *J* = 7.1 Hz), 6.99 (1H, d, *J* = 8.5 Hz), 6.82 (1H, s), 3.98 (3H, s), 3.66 (3H, s), 3.22 - 3.15 (4H, m), 2.16 (3H, d, *J* = 0.9 Hz), 1.98 - 1.92 (4H, m); **^{13}C NMR** (126 MHz, $CDCl_3$): δ ppm 163.3, 163.1, 157.4, 144.1, 136.8, 135.8, 133.2, 132.7, 127.2, 126.2, 121.8, 121.7, 121.7, 115.7, 111.5, 102.4, 56.0, 51.6, 29.6, 24.8, 17.7; **HRMS**: *m/z* NSI+ [Found $(M+H)^+$ 392.1958 $C_{23}H_{25}N_3O_3$ requires M^+ 392.1896]; **HPLC**: Retention time (system A): t_R= 10.40 min. Purity: 91%.



1,3-Dimethyl-6-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (254) **129a** (100 mg, 0.53 mmol, 1 eq.) was dissolved in dry DMF (2 mL) under argon. Cs_2CO_3 (690 mg, 2.12 mmol, 4 eq.) and 1,4 diiodobutane (198 mg, 0.64 mmol, 1.2 eq.) was added and the resulting suspension was heated to 80 °C overnight. After cooling to RT the reaction was diluted with H_2O (20 mL) and extracted with EtOAc (3×50 mL). The organic fractions were pooled, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give a residue which was purified by column chromatography (acetone:hexane ; 1:9 – 3:7) to give the title compound (73 mg, 0.30 mmol, 57%) as a bright yellow solid: **mp** 136 - 137 °C (acetone-hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.40 (1H, s), 7.15 (1H, d, *J* = 8.8 Hz), 6.77 (1H, dd, *J* = 9.1, 2.5 Hz), 6.51 (1H, d, *J* = 2.5 Hz), 3.30 - 3.18 (4H, m), 1.97 (4H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 162.2, 143.6, 135.4, 130.5, 130.2, 121.9, 114.9, 114.8, 108.1, 48.0, 29.6, 25.5, 17.9.

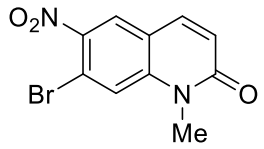


7-Bromo-1,3-dimethyl-6-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (255) **254** (50 mg, 0.21 mmol, 1 eq.) was dissolved in dry DMF (2 mL) and freshly recrystallized NBS (39 mg, .22 mmol, 1.05 eq.) was added in 1 portion and stirred overnight. Further NBS (78 mg, 0.42 mmol, 2.1 eq.) was added and the solution was stirred for a further 24 hours. The solution was diluted with H_2O (10 mL) and extracted with EtOAc (3×20 mL). The organic fractions were pooled, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give a residue which was purified by column chromatography (acetone:hexane 1:9 – 3:7) to give the title compound (8 mg, 0.025 mmol, 12%) as a bright yellow solid: **mp** 105 - 106 °C (acetone-hexane); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.06 (1H, d, *J* = 1.3 Hz), 7.17 (2H, app. s), 3.66 (3H, s), 3.29 - 3.21 (4H, m), 2.23 (3H, d, *J* = 1.3 Hz), 1.91 (4H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 162.1, 134.4, 131.5, 121.1, 120.2, 115.6, 113.6, 51.8, 30.1, 24.9, 18.2.

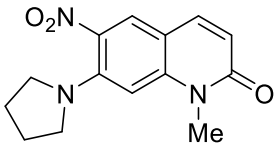


7-Bromo-6-nitroquinolin-2(1*H*)-one (257) Prepared according to general procedure G from **246** (2.5 g, 10.2 mmol) to give the title compound (1.80 g, 6.79 mmol, 66%) as a black solid that

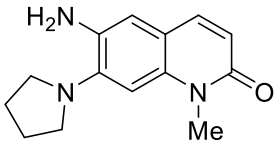
was used without further purification Relevant **^{1}H NMR** (500 MHz, DMSO-d_6): δ 12.21 (1 H, br. s.), 8.58 (1H, s), 8.05 (1H, d, *J* = 9.8 Hz), 7.66 (1H, s), 6.70 (1H, dd, *J* = 9.6, 1.7 Hz).



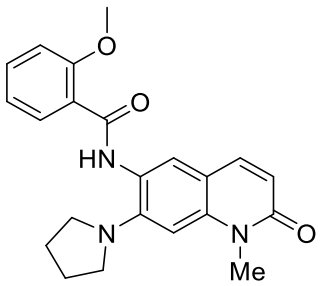
7-Bromo-1-methyl-6-nitroquinolin-2(1*H*)-one (258) Crude **257** (1.2 g, 4.46 mmol, 1 eq.) methylated according to general procedure B. Purification by column chromatography (acetone:hexane 1:20 – 2:8) followed by trituration of product fractions with cold acetone gave the title compound (213 mg, 0.76 mmol, 17%) as a pale yellow solid: **mp** 227 – 229 °C (acetone-hexane); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.24 (1H, s), 7.71 (1H, d *J* = 9.6 Hz), 6.85 (1H, d, *J* = 9.6 Hz), 3.75 (3H, s); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 161.5, 143.1, 142.7, 137.7, 126.4, 124.3, 120.5, 119.3, 117.0, 29.4.



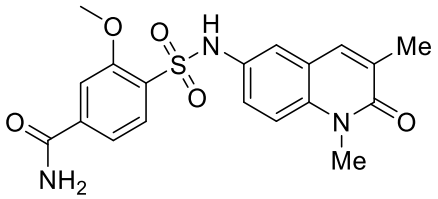
1-Methyl-6-nitro-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (259) – 258 (100 mg, 0.35 mmol, 1 eq.), CuI (7 mg, 0.04 mmol, 0.1 eq.) and pyrrolidine (125 mg, 1.75 mmol, 5 eq.) were dissolved in dry DMF and heated at 80 °C for 3 hours. After cooling to RT the solution was poured onto ice (~50 g) stirred for 10 mins and filtered to give the title compound (74 mg, 0.27 mmol, 77%) as a brown solid: **mp** 212 °C (decomposed); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 8.01 (1H, s), 7.56 (1H, d, *J* = 9.5 Hz), 6.58 - 6.47 (2H, m), 3.67 (3H, s), 3.42 - 3.28 (4H, m), 2.17 – 1.98 (4H, m); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 162.8, 143.8, 143.5, 133.4, 127.7, 118.4, 110.7, 98.4, 50.9, 29.4, 25.8.



6-Amino-1-methyl-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (260) Prepared by general procedure D from **259** (50 mg, 0.18 mmol). Compound precipitated on basification to pH 11 and was filtered, washed with H_2O and dried to give the title compound (34 mg, 0.14 mmol, 77%) as a dark red solid: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.41 (1H, d, *J* = 9.3 Hz), 6.75 (1H, s), 6.74 (1H, s), 6.46 (1H, d, *J* = 9.3 Hz), 3.67 - 3.55 (5H, m), 3.24 - 3.10 (4H, m), 1.95 – 1.93 (4H, m); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 162.3, 142.4, 137.8, 135.5, 134.6, 118.7, 115.8, 113.4, 102.5, 50.6, 29.4, 24.4.



2-Methoxy-*N*-(1-methyl-2-oxo-7-(pyrrolidin-1-yl)-1,2-dihydroquinolin-6-yl)benzamide (261) Prepared by general procedure E from **260** (20 mg, 0.083 mmol). Purified by column chromatography (acetone:hexane 2:8) to give the title compound (31 mg, 0.082 mmol, quant) as a pale yellow solid: **mp** 209 - 211 °C (acetone-hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 10.11 (1H, s), 8.41 (1H, s), 8.39 (1H, dd, *J* = 7.9, 1.9 Hz), 7.65 (1H, d, *J* = 9.5 Hz), 7.55 (1H, td, *J* = 8.8, 1.9 Hz), 7.20 (1H, t, *J* = 7.9 Hz), 7.09 (1H, d, *J* = 8.2 Hz), 6.89 (1H, s), 6.59 (1H, d, *J* = 9.5 Hz), 4.08 (3H, s), 3.73 (3H, s), 3.35 - 3.30 (4H, m), 2.07 - 2.02 (4H, m); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 163.4, 162.7, 157.4, 145.6, 138.9, 138.0, 133.3, 132.8, 125.5, 123.3, 121.7, 121.6, 118.7, 115.2, 111.5, 102.0, 56.0, 51.3, 29.3, 24.9; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 376.1662, $C_{22}H_{23}N_3O_3$ requires $(M-H)^-$ 376.1661]; **HPLC**: Retention time (system A): t_R= 9.97 min. Purity: >95%.

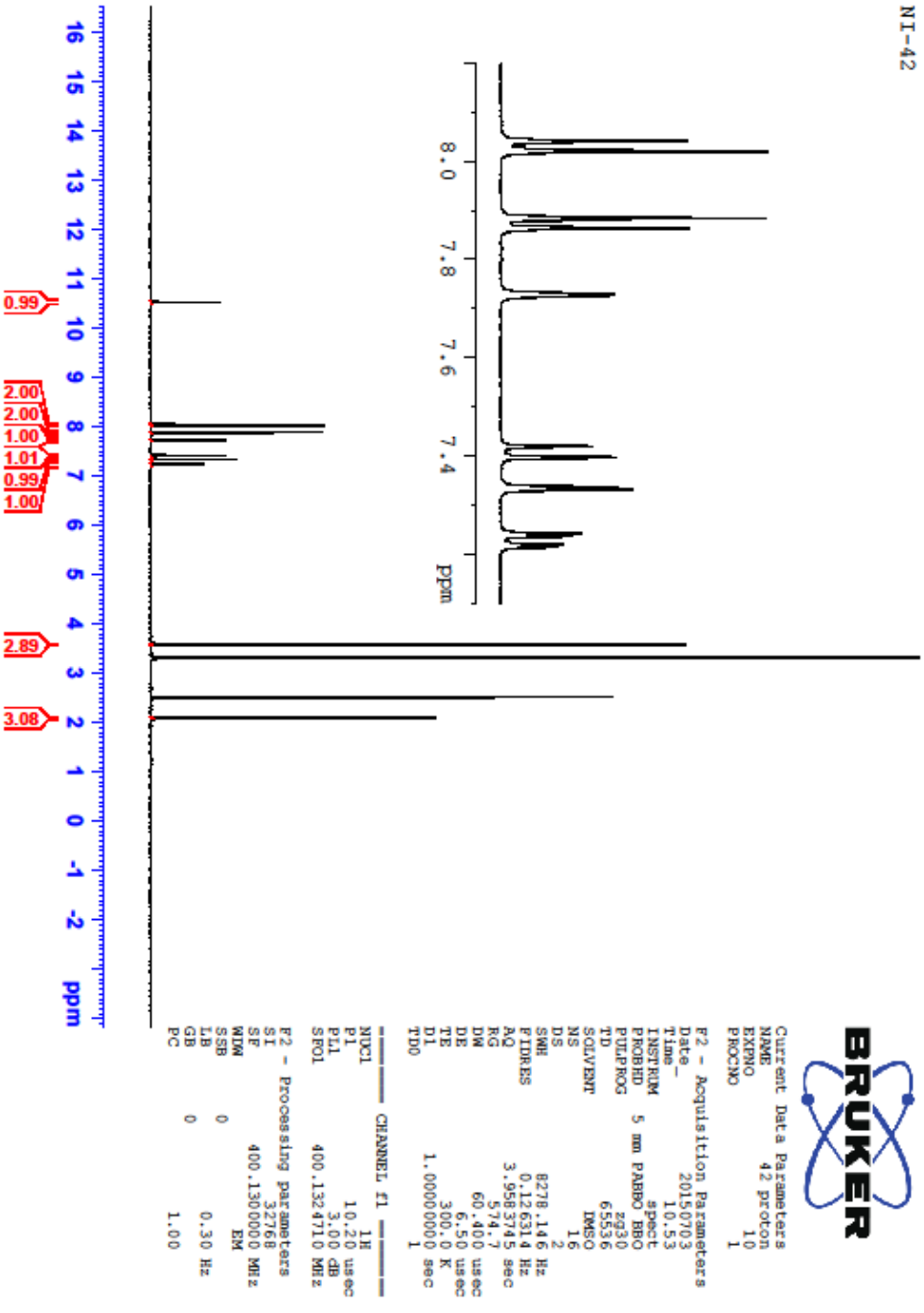


4-(*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)sulfamoyl)-3-methoxybenzamide (262) To a suspension of **NI-57** (45 mg, 0.12 mmol, 1 eq.) and potassium carbonate (65 mg, 0.37 mmol, 3 eq.) in dry DMSO (250 µL) was added hydrogen peroxide (30% w/w aqueous solution, 0.24 mmol, 2 eq.) in 1 portion and the resulting suspension was stirred overnight. Water (10 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic extracts were pooled, washed with brine (20 mL), dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure to give the title compound (41 mg, 0.10 mmol, 87%) as a colourless solid: **mp** 285 °C (ethyl acetate); **^{1}H NMR** (500 MHz, DMSO-d_6): δ ppm 10.12 (1H, s), 8.11 (1H, s), 7.78 (1H, d, *J* = 8.2 Hz), 7.69 (1H, s), 7.62 - 7.51 (2H, m), 7.45 (1H, dd, *J* = 8.0, 1.4 Hz), 7.36 (1H, d, *J* = 9.1 Hz), 7.31 (1H, d, *J* = 2.5 Hz), 7.27 (1H, dd, *J* = 9.1, 2.2 Hz), 3.97 (3H, s), 3.55 (3H, s), 2.08 (3H, s); **^{13}C NMR** (126 MHz, DMSO-d_6): δ ppm 166.4, 161.3, 156.1, 140.3, 135.7, 135.0, 131.6, 130.3, 129.8, 128.4, 122.6, 120.2, 119.0, 118.6, 115.2, 111.6, 56.3, 29.3, 17.3; **HRMS**: *m/z* ESI- [Found $(M-H)^-$ 400.0968 $C_{19}H_{19}N_3O_5S$ requires $(M-H)^-$ 400.0967]; **HPLC**: Retention time (system A): t_R= 6.35 min. Purity: >95%.

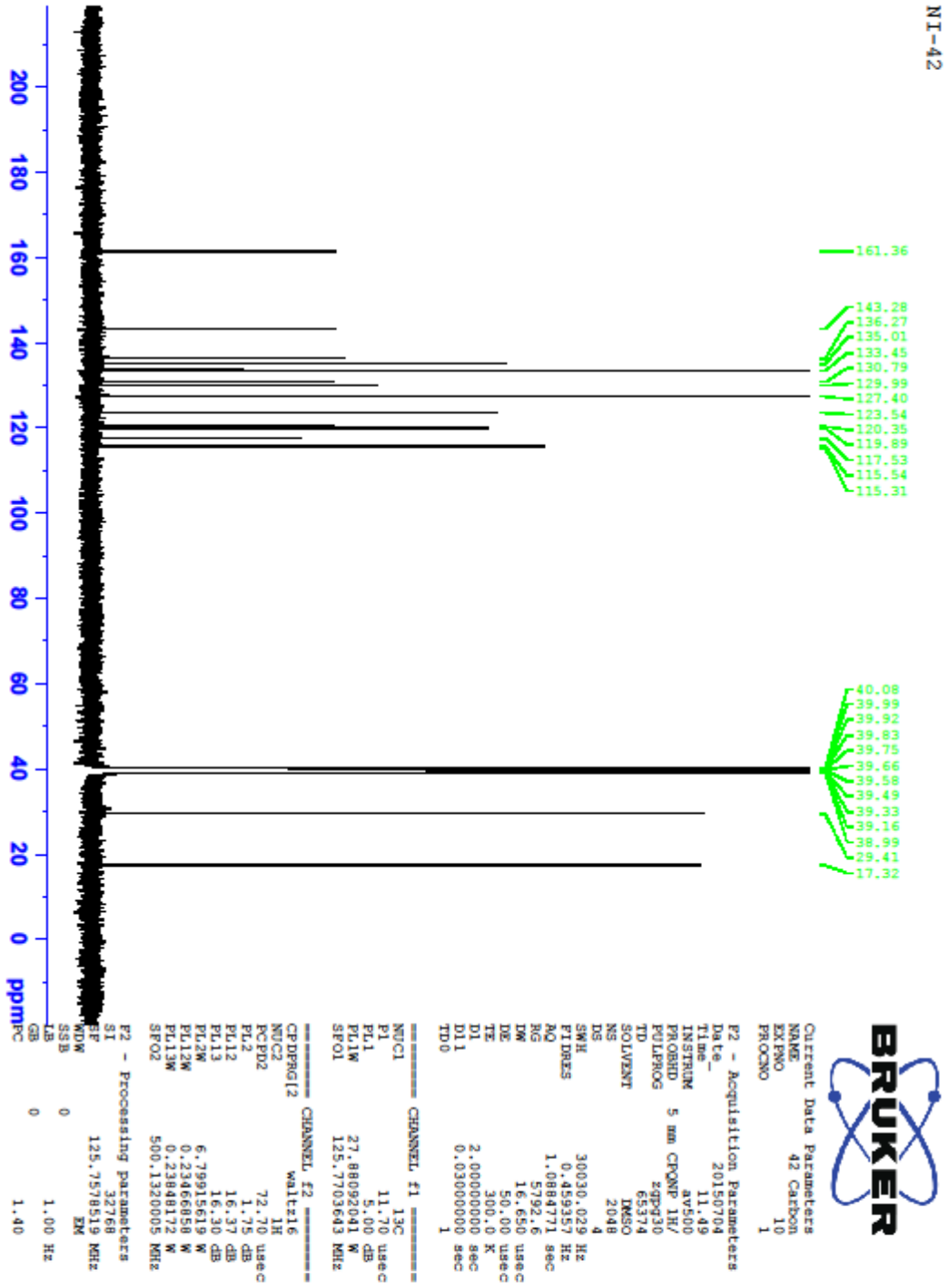
9. Appendices

9.1 Spectra for NI-57 and NI-42

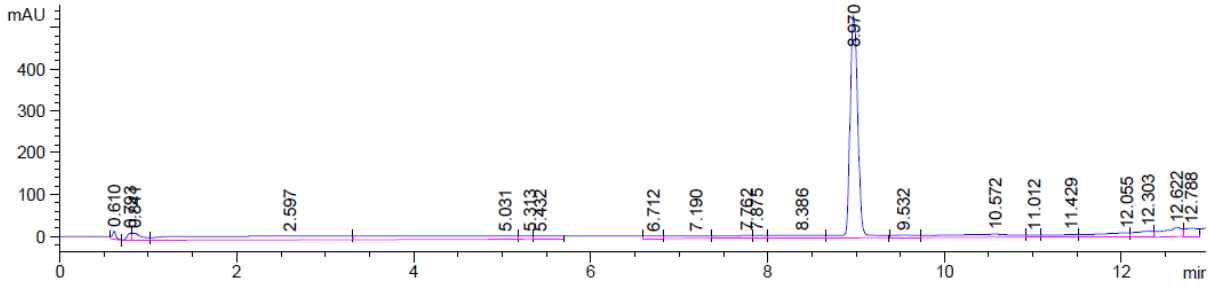
9.1.1 NI-42 ^{1}H NMR



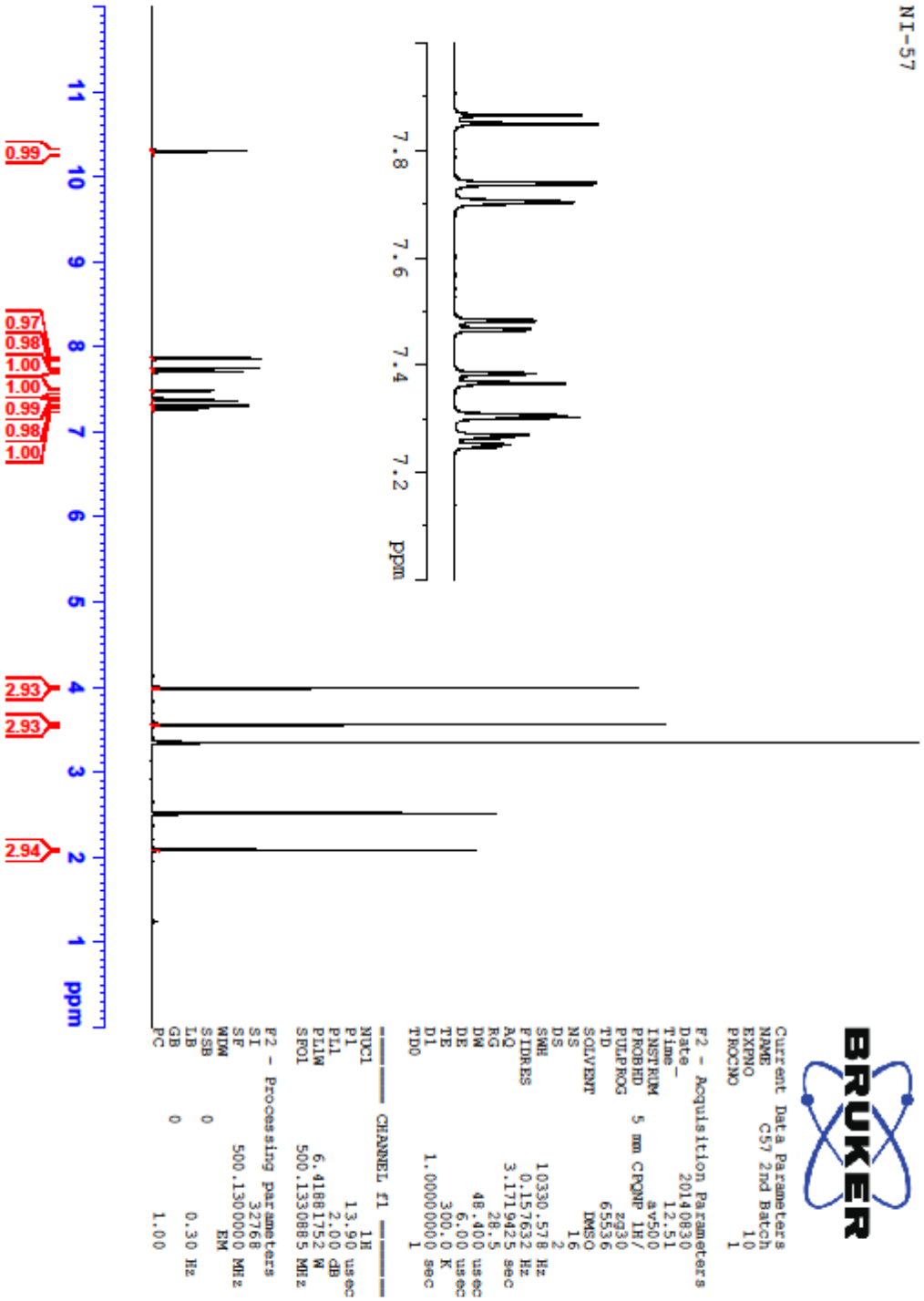
9.1.2 NI-42 ^{13}C NMR



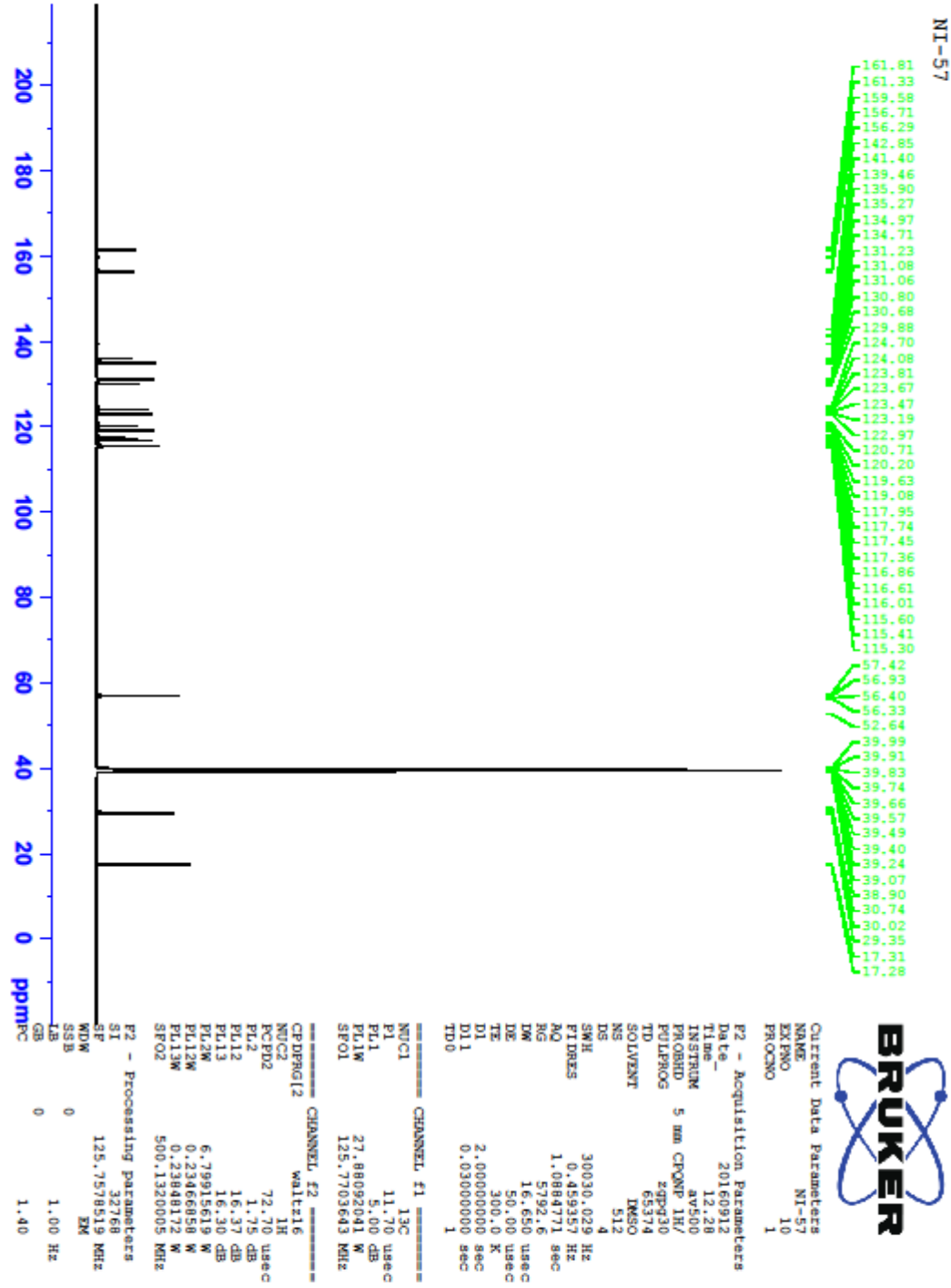
9.1.3 NI-42 HPLC



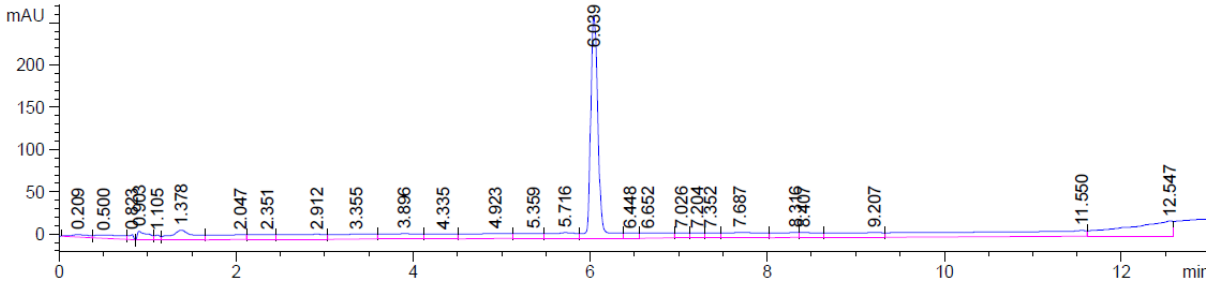
9.1.4 NI-57 ^{1}H NMR



9.1.5 NI-42 ^{13}C NMR



9.1.6 NI-57 HPLC



9.2 DSF (Thermal Shift Data) for bromonome wide screeining of NI-42, NI-57 and 164

9.2.1 NI-42

BRD	T_m (°C)	BRD	T_m (°C)	BRD	T_m (°C)
ASH1L	0.5	BRDT92)	-0.3	PB1(4)	0.0
ATAD2	-0.4	BRPF1A	0.2	PB1(5)	0.2
BAZ1A	-0.9	BRPF1B	7.8	PB1(6)	0.0
BAZ1B	0.2	BRPF3	2.4	PCAF	0.3
BAZ2A	-0.8	BRWD3(2)	0.5	PHIP(2)	-0.8
BAZ2B	-0.5	CECR2	0.4	SMARCA2	0.2
BRD1	4.2	CREBBP	0.5	SMARCA4	-0.3
BRD2(1)	0.4	EP300	0.8	SP140	-0.4
BRD2(2)	-0.2	FALZ	0.3	TAF1(2)	-0.6
BRD3(1)	0.6	GCN5L2	-0.3	TAF1(1)	0.0
BRD3(2)	-0.2	ATAD2B	-0.1	TAF1L(1)	0.7
BRD4(1)	0.4	SP140L	-0.2	TAF1L(2)	-1.9
BRD4(2)	0.3	MLL	0.0	TIF1-bromo	0.4
BRD7	4.8	PB1(1)	-0.1	TIF1-phd-bromo	0.3
BRD9	1.8	PB1(2)	0.0	TRIM28	-1.0
BRDT(1)	0.0	PB1(3)	0.2	WDR9(2)	-0.2

9.2.2 NI 57

BRD	T_m (°C)	BRD	T_m (°C)	BRD	T_m (°C)

ASH1L	-0.5	BRDT(2)	0.2	PB1(4)	0.6
ATAD2	-0.5	BRPF1A	0.9	PB1(5)	0.3
BAZ1A	-0.3	BRPF1B	10.7	PB1(6)	0.3
BAZ1B	0	BRPF3	5.3	PCAF	0.5
BAZ2A	-0.4	BRWD3(2)	1.5	PHIP(2)	-0.4
BAZ2B	0	CECR2	0.3	SMARCA2	0.5
BRD1	5.6	CREBBP	1.1	SMARCA4	0.2
BRD2(1)	0.4	EP300	1.1	SP140	0.9
BRD2(2)	0	FALZ	0.3	TAF1(2)	0.5
BRD3(1)	0.8	GCN5L2	0.3	TAF1(1)	0.4
BRD3(2)	0.2	ATAD2B	-0.1	TAF1L(1)	0.7
BRD4(1)	0.8	SP140L	0.5	TAF1L(2)	0.7
BRD4(2)	-0.1	MLL	0.3	TIF1-bromo	2
BRD7	6.6	PB1(1)	0.5	TIF1-phd-bromo	1.6
BRD9	3.1	PB1(2)	0.4	TRIM28	0.8
BRDT(1)	0.2	PB1(3)	0.1	WDR9(2)	0.7

9.2.3 Compound 164

BRD	T_m (°C)	BRD	T_m (°C)	BRD	T_m (°C)
ASH1L	0.8	BRDT(2)	1	PB1(4)	1
ATAD2	0.2	BRPF1A	0.6	PB1(5)	0.8
BAZ1A	1.1	BRPF1B	10.9	PB1(6)	0.6
BAZ1B	1	BRPF3	5.6	PCAF	0.9
BAZ2A	-0.3	BRWD3(2)	0.7	PHIP(2)	0.2
BAZ2B	0.2	CECR2	0.6	SMARCA2	0.3
BRD1	6	CREBBP	2.2	SMARCA4	0.6
BRD2(1)	2.2	EP300	2.2	SP140	1.5
BRD2(2)	0.5	FALZ	1.9	TAF1(2)	0.7
BRD3(1)	2.3	GCN5L2	1	TAF1(1)	0.6
BRD3(2)	0.9	ATAD2B	0.4	TAF1L(1)	1.1
BRD4(1)	2.6	SP140L	1.5	TAF1L(2)	1.7
BRD4(2)	0.3	MLL	1	TIF1-bromo	2.5
BRD7	8.9	PB1(1)	0.9	TIF1-phd-bromo	1.5
BRD9	6.5	PB1(2)	0.7	TRIM28	0.3
BRDT(1)	1.1	PB1(3)	0.3	WDR9(2)	0.8

9.3 Data from NCI-60 Cancer panel, AZ CLIMB Panel and Cerep Safety Panel

9.3.1 NCI-60 Panel for NI-57

Conc (M)	Cancer Type	Cell Line	Growth Percentage (%)

0.00001	Leukemia	CCRF-CEM	89.9
0.00001	Leukemia	HL-60(TB)	95.7
0.00001	Leukemia	K-562	77.0
0.00001	Leukemia	MOLT-4	86.3
0.00001	Leukemia	RPMI-8226	79.3
0.00001	Leukemia	SR	82.0
0.00001	Non-Small Cell Lung Cancer	A549/ATCC	87.5
0.00001	Non-Small Cell Lung Cancer	EKVX	89.7
0.00001	Non-Small Cell Lung Cancer	HOP-62	88.6
0.00001	Non-Small Cell Lung Cancer	HOP-92	91.8
0.00001	Non-Small Cell Lung Cancer	NCI-H226	85.7
0.00001	Non-Small Cell Lung Cancer	NCI-H23	92.8
0.00001	Non-Small Cell Lung Cancer	NCI-H322M	93.9
0.00001	Non-Small Cell Lung Cancer	NCI-H460	102.6
0.00001	Non-Small Cell Lung Cancer	NCI-H522	73.3
0.00001	Colon Cancer	COLO 205	114.7
0.00001	Colon Cancer	HCC-2998	96.7
0.00001	Colon Cancer	HCT-116	95.9
0.00001	Colon Cancer	HCT-15	106.6
0.00001	Colon Cancer	HT29	100.5
0.00001	Colon Cancer	KM12	92.7
0.00001	Colon Cancer	SW-620	100.0
0.00001	CNS Cancer	SF-268	101.7
0.00001	CNS Cancer	SF-295	90.6
0.00001	CNS Cancer	SF-539	103.7
0.00001	CNS Cancer	SNB-19	105.2

0.00001	CNS Cancer	SNB-75	82.0
0.00001	CNS Cancer	U251	92.9
0.00001	Melanoma	LOX IMVI	95.4
0.00001	Melanoma	MALME-3M	93.8
0.00001	Melanoma	M14	97.9
0.00001	Melanoma	MDA-MB-435	96.2
0.00001	Melanoma	SK-MEL-2	98.5
0.00001	Melanoma	SK-MEL-28	108.2
0.00001	Melanoma	SK-MEL-5	93.0
0.00001	Melanoma	UACC-257	92.1
0.00001	Melanoma	UACC-62	82.9
0.00001	Ovarian Cancer	IGROV1	97.0
0.00001	Ovarian Cancer	OVCAR-3	103.4
0.00001	Ovarian Cancer	OVCAR-4	77.0
0.00001	Ovarian Cancer	OVCAR-5	99.3
0.00001	Ovarian Cancer	OVCAR-8	94.8
0.00001	Ovarian Cancer	NCI/ADR-RES	97.2
0.00001	Ovarian Cancer	SK-OV-3	106.2
0.00001	Renal Cancer	786-0	96.9
0.00001	Renal Cancer	A498	107.6
0.00001	Renal Cancer	ACHN	95.6
0.00001	Renal Cancer	CAKI-1	85.2
0.00001	Renal Cancer	RXF 393	101.8
0.00001	Renal Cancer	SN12C	92.1
0.00001	Renal Cancer	TK-10	90.8
0.00001	Renal Cancer	UO-31	75.2

0.00001	Prostate Cancer	PC-3	84.0
0.00001	Prostate Cancer	DU-145	99.8
0.00001	Breast Cancer	MCF7	83.4
0.00001	Breast Cancer	MDA-MB-231/ATCC	97.1
0.00001	Breast Cancer	HS 578T	97.2
0.00001	Breast Cancer	BT-549	99.3
0.00001	Breast Cancer	T-47D	77.2
0.00001	Breast Cancer	MDA-MB-468	76.2

Table 50. Data for tne NCI-60 cancer panel screen of **NI-57.** Work carried out at the National Institute of Health

9.3.2 AZ CLIMB Panel

Label, Species, cell line, GI_{50} (µM)	**NI-57**	**NI-42**
IT16707 Hu SW48 Prolif Cell Panel Mean GI50 (µM)	30	1.2
IT16718 Hu OCI-AML2 Prolif Cell Panel Mean GI50 (µM)	10	1.3
IT13873 Hu NUGC-4 Prolif Cell Panel Mean GI50 (µM)	30	1.6
IT13884 Hu HepG2 prolif Cell Panel Mean GI50 (µM)	30	2.0
IT16634 Hu COLO 205 Prolif Cell Panel Mean GI50 (µM)	30	2.6
IT13872 Hu OCUM-1 Prolif Cell Panel Mean GI50 (µM)	30	2.7
IT21518 Hu HUP-T4 Prolif cell panel Mean GI50 (µM)	30	3.0
IT16702 Hu LOVO Prolif Cell Panel Mean GI50 (µM)	30	4.2
IT17872 Hu C-99 Prolif Cell Panel Mean GI50 (µM)	30	4.3
IT19190 Hu 22Rv1 Prolif cell panel Mean GI50 (µM)	30	4.4
IT16706 Hu SK-CO-1 Prolif Cell Panel Mean GI50 (µM)	30	4.5
IT16716 Hu Nomo-1 Prolif Cell Panel Mean GI50 (µM)	10	4.6
IT16730 Hu MOLP-8 Profil Cell Panel Mean GI50 (µM)	10	5.1
IT21527 Hu QGP-1 Prolif cell panel Mean GI50 (µM)	30	5.2
IT13914 Hu AGS Prolif Cell Panel Mean GI50 (µM)	30	5.3

IT16710 Hu THP-1 Prolif Cell Panel Mean GI50 (µM)	10	5.7
IT21515 Hu Hs 766T Prolif cell panel Mean GI50 (µM)	30	6.1
IT13924 Hu SNU-5 Prolif Cell Panel Mean GI50 (µM)	30	6.3
IT16715 Hu SW948 Prolif Cell Panel Mean GI50 (µM)	30	6.3
IT16705 Hu RKO Prolif Cell Panel Mean GI50 (µM)	30	6.7
IT16647 Hu OCI-LY-19 Prolif Cell Panel Mean GI50 (µM)	10	6.8
IT16692 Hu KG-1 Prolif Cell Panel Mean GI50 (µM)	10	7.0
IT21521 Hu Panc02.03 Prolif cell panel Mean GI50 (µM)	30	7.5
IT16713 Hu SW403 Prolif Cell Panel Mean GI50 (µM)	30	7.6
IT17314 Hu HCC1187 Prolif Cell Panel Mean GI50 (µM)	30	7.7
IT16672 Hu NCI-H1437 Prolif Cell Panel Mean GI50 (µM)	30	8.1
IT16673 Hu IM-9 Profil Cell Panel Mean GI50 (µM)	10	8.2
IT21504 Hu AsPC-1 Prolif cell panel Mean GI50 (µM)	30	8.4
IT16711 Hu SW620 Prolif Cell Panel Mean GI50 (µM)	30	8.8
IT16704 Hu LS 180 Prolif Cell Panel Mean GI50 (µM)	30	9.9
IT18016 Hu MV-4-11 Prolif Cell Panel Mean GI50 (µM)	10	9.9
IT16709 Hu Molm 16 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16712 Hu MonoMac6 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16714 Hu Reh Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16729 Hu L-363 Profil Cell Panel Mean GI50 (µM)	10	10.0
IT16734 Hu RPMI-8226 Profil Cell Panel Mean GI50 (µM)	10	10.0
IT18014 Hu Hel92.1.7 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT18015 Hu Molm 13 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT18017 Hu RS411 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16630 Hu JEKO-1 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16643 Hu JVM-3 Prolifl Cell Panel Mean GI50 (µM)	10	10.0

IT16645 Hu MEC-1 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16646 Hu NAMALWA Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16648 Hu Raji Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16654 Hu Ramos Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16656 Hu CMK Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16657 Hu SC-1 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16664 Hu Jurkat Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16666 Hu K-562 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16668 Hu AMO-1 Profil Cell Panel Mean GI50 (µM)	10	10.0
IT16669 Hu ARH-77 Profil Cell Panel Mean GI50 (µM)	10	10.0
IT16670 Hu JJN-3 Profil Cell Panel Mean GI50 (µM)	10	10.0
IT16677 Hu U937 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16679 Hu WSU DLCL2 Prolif Cell Panel Mean GI50 (µM)	10	10.0
IT16662 Hu HT-29 Prolif Cell Panel Mean GI50 (µM)	30	12.3
IT17322 Hu MDA-MB-157 Prolif Cell Mean GI50 (µM)	30	12.4
IT13920 Hu SNU-1 Prolif Cell Panel Mean GI50 (µM)	30	12.4
IT13891 Hu SNU-878 Prolif Cell Panel Mean GI50 (µM)	30	12.7
IT21514 Hu HPAF-II Prolif cell panel Mean GI50 (µM)	30	12.7
IT13893 Hu SNU-449 Prolif Cell Panel Mean GI50 (µM)	30	12.8
IT13913 Hu 23132/87 Prolif Cell Panel Mean GI50 (µM)	30	13.4
IT18616 Hu KU-19-19 Prolif Cell Panel Mean GI50 (µM)	30	14.0
IT21525 Hu Panc10.05 Prolif cell panel Mean GI50 (µM)	30	14.1
IT13876 Hu BEL7405 Prolif Cell panel Mean GI50 (µM)	30	14.3
IT17316 Hu HCC1419 Prolif Cell Panel Mean GI50 (µM)	23.44	14.3
IT16641 Hu Calu-3 Prolif Cell Panel Mean GI50 (µM)	30	14.4
IT18618 Hu RT4 Prolif Cell Panel Mean GI50 (µM)	30	15.4

IT17876 Hu HRA-19 Prolif Cell Panel Mean GI50 (µM)	15.62	15.7
IT13908 Hu SNU-638 Prolif Cell Panel Mean GI50 (µM)	30	15.8
IT13919 Hu NCl-N87 Prolif Cell Panel Mean GI50 (µM)	30	16.2
IT16639 Hu Calu-6 Prolif Cell Panel Mean GI50 (µM)	30	16.4
IT17874 Hu CCK-81 Prolif Cell Panel Mean GI50 (µM)	30	16.4
IT16640 Hu DMS 114 Prolif Cell Panel Mean GI50 (µM)	14.67	16.6
IT19867 Hu LUDLU-1 Prolif cell panel Mean GI50 (µM)	30	16.7
IT19904 Hu RERF-LC-Sq1 Prolif Mean GI50 (µM)	16.57	17.2
IT13902 Hu HLE Prolif Cell panel Mean GI50 (µM)	30	17.2
IT13911 Hu SNU-484 Prolif Cell Panel Mean GI50 (µM)	30	17.2
IT13972 Hu Hep3B Prolif Cell Panel Mean GI50 (µM)	30	17.4
IT13907 Hu SNU-620 Prolif Cell Panel Mean GI50 (µM)	30	17.6
IT13910 Hu SNU-601 Prolif Cell Panel Mean GI50 (µM)	30	18.0
IT21513 Hu HPAC Prolif cell panel Mean GI50 (µM)	30	18.5
IT18636 Hu SW780 Prolif cell panel Mean GI50 (µM)	30	19.1
IT16674 Hu NCI-H1869 Prolif Cell Panel Mean GI50 (µM)	30	19.8
IT16652 Hu NIH:OVCAR-3 Prolif Mean GI50 (µM)	30	20.4
IT13900 Hu HuH-7 Prolif Cell Panel Mean GI50 (µM)	30	20.8
IT18641 Hu 5637 Prolif cell panel Mean GI50 (µM)	30	21.1
IT13898 Hu SNU-354 prolif Cell Panel Mean GI50 (µM)	30	21.6
IT16694 Hu NCI-H226 Prolif Cell Panel Mean GI50 (µM)	30	21.6
IT16650 Hu MDA-MB-231 Prolif CellPanel Mean GI50 (µM)	30	21.9
IT21517 Hu HUP-T3 Prolif cell panl Mean GI50 (µM)	30	22.1
IT19899 Hu NCI-H2286 Prolif cell panel Mean GI50 (µM)	30	22.1
IT14567 Hu NUGC-3 Prolif Cell Panel Mean GI50 (µM)	30	22.9
IT14028 Hu PAMC82 Prolif Cell Panel Mean GI50 (µM)	30	22.9

IT16633 Hu HCT 116 Prolif Cell Panel Mean GI50 (µM)	30	23.0
IT16632 Hu HX147 Prolif cell panel Mean GI50 (µM)	30	23.5
IT18627 Hu BFTC-905 Prolif cell panel Mean GI50 (µM)	30	23.6
IT19856 Hu EBC-1 Prolif cell panel Mean GI50 (µM)	30	23.8
IT13894 Hu SNU-398 prolif Cell Panel Mean GI50 (µM)	30	24.1
IT21526 Hu PANC-89 Prolif cell panel Mean GI50 (µM)	30	24.4
IT21522 Hu Panc03.27 Prolif cell panel Mean GI50 (µM)	30	24.9
IT18619 Hu SCaBER Prolif cell panel Mean GI50 (µM)	30	25.7
IT21510 Hu Capan-2 Prolif cell panel Mean GI50 (µM)	30	25.7
IT13896 Hu SNU-368 Prolif Cell Panel Mean GI50 (µM)	30	26.3
IT18628 Hu HT1197 Prolif cell panel Mean GI50 (µM)	30	26.5
IT17984 Hu OE33 Prolif Cell Panel Mean GI50 (µM)	30	26.6
IT17321 Hu JIMT-1 Prolif Cell Panel Mean GI50 (µM)	30	26.9
IT16637 Hu NCI-H1299 Prolif Cell Panel Mean GI50 (µM)	30	27.2
IT16708 Hu SW480 Prolif Cell Panel Mean GI50 (µM)	30	27.2
IT17323 Hu ZR-75-1 Prolif Cell Panel Mean GI50 (µM)	30	27.3
IT18640 Hu VM-CUB-1 Prolif cell panel Mean GI50 (µM)	30	27.5
IT13887 Hu SNU-886 Prolif Cell Panel Mean GI50 (µM)	30	27.9
IT16689 Hu HCT-8 Prolif Cell Panel Mean GI50 (µM)	30	28.5
IT17873 Hu CC20 Prolif Cell Panel Mean GI50 (µM)	30	28.7
IT19859 Hu HCC-15 Prolif cell panel Mean GI50 (µM)	30	28.7
IT21519 Hu KP-4 Prolif cell panel Mean GI50 (µM)	30	28.8
IT19192 Hu PNT1A Prolif cell panel Mean GI50 (µM)	30	29.9
IT18642 Hu 647V Prolif cell panel Mean GI50 (µM)	30	30.0
IT16697 Hu COLO 320DM Prolif Mean GI50 (µM)	30	30.0
IT16698 Hu NCI-H358 Prolif Cell Panel Mean GI50 (µM)	30	30.0

IT16699 Hu NCI-H522 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16701 Hu NCI-H838 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16703 Hu PC9 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13874 Hu MKN74 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13875 Hu BEL7404 Prolif cell panel Mean GI50 (µM)	30	30.0
IT13878 Hu HCCC9810 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13880 Hu MKN1 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13881 Hu IM95m Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13883 Hu SK-HEP-1 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13888 Hu SNU-761 prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13889 Hu MHCC97L Prolif Cell panel Mean GI50 (µM)	30	30.0
IT13890 Hu QGY7703 Prolif Cell panel Mean GI50 (µM)	30	30.0
IT13892 Hu SNU-739 prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17311 Hu BT-20 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13897 Hu SMMC7721 Prolif Cell panel Mean GI50 (µM)	30	30.0
IT17312 Hu BT-549 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17313 Hu CAMA-1 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13901 Hu HuH-1 prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17315 Hu HCC1395 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17317 Hu HCC1569 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13903 Hu HLF Prolif Cell panel Mean GI50 (µM)	30	30.0
IT17318 Hu HCC1806 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17319 Hu HCC1937 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17320 Hu HCC1954 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13909 Hu SNU-668 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17324 Hu T47D Prolif Cell Panel Mean GI50 (µM)	30	30.0

IT13912 Hu SNU-216 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17326 Hu MDA-MB-436 Prolif Cell Mean GI50 (µM)	30	30.0
IT17327 Hu SUM52PE Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17328 Hu MDA-MB-453 Prolif Cell Mean GI50 (µM)	26.72	30.0
IT13915 Hu GTL-16 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13916 Hu HGC27 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13917 Hu KATO III Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17875 Hu HCA-7 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT17983 Hu OE19 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT13925 Hu SNU-16 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT14575 Hu HS746T Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT18615 Hu J82 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16631 Hu A549 Prolif cell panel Mean GI50 (µM)	30	30.0
IT18617 Hu RT112/84 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16635 Hu HCT-15 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16636 Hu Calu-1 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT18629 Hu HT1376 Prolif cell panel Mean GI50 (µM)	30	30.0
IT18630 Hu 1A6 [PTA-556] Prolif panel Mean GI50 (µM)	30	30.0
IT18633 Hu MGH-U3 Prolif cell panel Mean GI50 (µM)	30	30.0
IT18634 Hu 97-7 Prolif cell panel Mean GI50 (µM)	30	30.0
IT18635 Hu T24 Prolif cell panel Mean GI50 (µM)	30	30.0
IT18637 Hu SW1710 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16644 Hu LNCaP clone FGC Prolif Mean GI50 (µM)	30	30.0
IT18638 Hu TCC-SUP Prolif cell panel Mean GI50 (µM)	30	30.0
IT18639 Hu UM-UC-3 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16649 Hu MCF7 Cell Panel Mean GI50 (µM)	30	30.0

IT19191 Hu LNCap-CasRes(AZ) cell panel Mean GI50 (µM)	30	30.0
IT16651 Hu NCI-H23 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16653 Hu MIA PaCa-2 Prolif CellPanel Mean GI50 (µM)	30	30.0
IT19858 Hu HARA Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16655 Hu PANC-1 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT19861 Hu HCC95 Prolif cell panel Mean GI50 (µM)	30	30.0
IT19864 Hu LK-2 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16658 Hu SK-BR-3 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT19897 Hu NCI-H2170 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16663 Hu MDA-MB-468 Prolif CellPanel Mean GI50 (µM)	30	30.0
IT19900 Hu NCI-H520 Prolif cell panel Mean GI50 (µM)	30	30.0
IT19901 Hu NCI-H596 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16665 Hu NCI-H460 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT19902 Hu NCI-H647 Prolif cell panel Mean GI50 (µM)	30	30.0
IT19903 Hu RERF-LC-AI Prolif Mean GI50 (µM)	30	30.0
IT16667 Hu NCI-H460 dnp53 Prolif Mean GI50 (µM)	30	30.0
IT19905 Hu SK-MES-1 Prolif cell panel Mean GI50 (µM)	30	30.0
IT19906 Hu SW 900 Prolif cell panel Mean GI50 (µM)	30	30.0
IT21507 Hu BxPC-3 Prolif cell panel Mean GI50 (µM)	30	30.0
IT21509 Hu Capan-1 Prolif cell panel Mean GI50 (µM)	30	30.0
IT21511 Hu CFPAC-1 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16675 Hu MCF7/mdr+ Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16678 Hu PC-3 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16680 Hu NCI-H1703 Prolif Cell Panel Mean GI50 (µM)	20.65	30.0
IT16681 Hu NCI-H526 Prolif Cell Panel Mean GI50 (µM)	10.41	30.0
IT16684 Hu NCI-H1793 Prolif Cell Panel Mean GI50 (µM)	30	30.0

IT16686 Hu DU 145 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16687 Hu A2058 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT21523 Hu Panc04.03 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16690 Hu NCI-H1975 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT16691 Hu NCI-H2085 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT21529 Hu SU.86.86 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16693 Hu NCI-H2126 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT21530 Hu SW1990 Prolif cell panel Mean GI50 (µM)	30	30.0
IT21531 Hu T3M-4 Prolif cell panel Mean GI50 (µM)	30	30.0
IT16695 Hu NCI-H2291 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT21533 Hu YAPC Prolif cell panel Mean GI50 (µM)	30	30.0
IT16696 Hu NCI-H322 Prolif Cell Panel Mean GI50 (µM)	30	30.0
IT21927 Hu Panc08.13 Prolif cell panel Mean GI50 (µM)	30	30.0

Table 51. Data from the AZ CLIMB cancer panel screen of **NI-57** and **NI-42.** Work carried out at AZ. '10' indicates a GI_{50} >10 µM, '30' indicates a GI_{50} >30 µM.

9.4 Cerep Panel

Assay	Test Concentration (M)	% Inhibition of Control Specific Binding	Reference Compound
norepinephrine transporter (h) (antagonist radioligand)	1.0E-05	39	protriptyline
A1 (h) (antagonist radioligand)	1.0E-05	38	DPCPX
kappa (KOP) (agonist radioligand)	1.0E-05	20	U 50488
CB1 (h) (agonist radioligand)	1.0E-05	13	CP 55940
MT1 (ML1A) (h) (agonist radioligand)	1.0E-05	13	melatonin
EP4 (h) (agonist radioligand)	1.0E-05	12	PGE2
D2S (h) (antagonist radioligand)	1.0E-05	10	(+)butaclamol
NTS1 (NT1) (h) (agonist radioligand)	1.0E-05	10	neurotensin
5-HT5a (h) (agonist radioligand)	1.0E-05	8	serotonin
Cl- channel (GABA-gated) (antagonist radioligand)	1.0E-05	8	picrotoxinin
NK3 (h) (antagonist radioligand)	1.0E-05	7	SB 222200
Na+ channel (site 2) (antagonist radioligand)	1.0E-05	6	veratridine
NOP (ORL1) (h) (agonist radioligand)	1.0E-05	5	nociceptin
5-HT2B (h) (agonist radioligand)	1.0E-05	5	(±)DOI
mu (MOP) (h) (agonist radioligand)	1.0E-05	4	DAMGO
5-HT7 (h) (agonist radioligand)	1.0E-05	4	serotonin

dopamine transporter (h) (antagonist radioligand)	1.0E-05	4	BTCP
CCK1 (CCKA) (h) (agonist radioligand)	1.0E-05	3	CCK-8s
delta 2 (DOP) (h) (agonist radioligand)	1.0E-05	3	DPDPE
5-HT6 (h) (agonist radioligand)	1.0E-05	3	serotonin
Ca2+ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)	1.0E-05	3	D 600
B2 (h) (agonist radioligand)	1.0E-05	2	NPC 567
MC4 (h) (agonist radioligand)	1.0E-05	2	NDP-alpha -MSH
A3 (h) (agonist radioligand)	1.0E-05	0	IB-MECA
ETA (h) (agonist radioligand)	1.0E-05	0	endothelin-1
5-HT1A (h) (agonist radioligand)	1.0E-05	0	8-OH-DPAT
alpha 1 (non-selective) (antagonist radioligand)	1.0E-05	-2	prazosin
CCR1 (h) (agonist radioligand)	1.0E-05	-2	MIP-1alpha
A2A (h) (agonist radioligand)	1.0E-05	-3	NECA
H1 (h) (antagonist radioligand)	1.0E-05	-4	pyrilamine
M3 (h) (antagonist radioligand)	1.0E-05	-4	4-DAMP
5-HT1B (antagonist radioligand)	1.0E-05	-4	serotonin
KV channel (antagonist radioligand)	1.0E-05	-4	alpha -dendrotoxin
beta 1 (h) (agonist radioligand)	1.0E-05	-5	atenolol
5-HT3 (h) (antagonist radioligand)	1.0E-05	-5	MDL 72222
beta 2 (h) (agonist radioligand)	1.0E-05	-7	ICI 118551
CXCR2 (IL-8B) (h) (agonist radioligand)	1.0E-05	-7	IL-8

V1a (h) (agonist radioligand)	1.0E-05	-7	[d(CH2)51,Tyr(Me)2]-AVP
SKCa channel (antagonist radioligand)	1.0E-05	-7	apamin
AT1 (h) (antagonist radioligand)	1.0E-05	-8	saralasin
GAL2 (h) (agonist radioligand)	1.0E-05	-10	galanin
GABA (non-selective) (agonist radioligand)	1.0E-05	-11	GABA
Y2 (h) (agonist radioligand)	1.0E-05	-11	NPY
5-HT2A (h) (antagonist radioligand)	1.0E-05	-11	ketanserin
VPAC1 (VIP1) (h) (agonist radioligand)	1.0E-05	-11	VIP
alpha 2 (non-selective) (antagonist radioligand)	1.0E-05	-12	yohimbine
D1 (h) (antagonist radioligand)	1.0E-05	-12	SCH 23390
sst (non-selective) (agonist radioligand)	1.0E-05	-12	somatostatin-14
5-HT transporter (h) (antagonist radioligand)	1.0E-05	-12	imipramine
BZD (central) (agonist radioligand)	1.0E-05	-13	diazepam
NK2 (h) (agonist radioligand)	1.0E-05	-13	[Nleu10]-NKA (4-10)
M2 (h) (antagonist radioligand)	1.0E-05	-17	methoctramine
Y1 (h) (agonist radioligand)	1.0E-05	-17	NPY
M1 (h) (antagonist radioligand)	1.0E-05	-21	pirenzepine
H2 (h) (antagonist radioligand)	1.0E-05	-24	cimetidine

10. References

1. Hansen, J. C., Human mitotic chromosome structure: what happened to the 30-nm fibre? *The EMBO Journal* **2012,** *31* (7), 1621-1623.

2. Dawson, Mark A.; Kouzarides, T., Cancer Epigenetics: From Mechanism to Therapy. *Cell 150* (1), 12-27.

3. Bayarsaihan, D., Epigenetic Mechanisms in Inflammation. *Journal of Dental Research* **2011,** *90* (1), 9-17.

4. Szyf, M., Epigenetics, a key for unlocking complex CNS disorders? Therapeutic implications. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* **2015,** *25* (5), 682-702.

5. Dennis, C., Epigenetics and disease: Altered states. *Nature* **2003,** *421* (6924), 686-688.

6. Egger, G.; Liang, G.; Aparicio, A.; Jones, P. A., Epigenetics in human disease and prospects for epigenetic therapy. *Nature* **2004,** *429* (6990), 457-463.

7. Bedford, M. T.; Clarke, S. G., Protein Arginine Methylation in Mammals: Who, What, and Why. *Molecular Cell* **2009,** *33* (1), 1-13.

8. Allfrey, V. G.; Faulkner, R.; Mirsky, A. E., ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS. *Proceedings of the National Academy of Sciences of the United States of America* **1964,** *51*, 786-94.

9. Bannister, A. J.; Kouzarides, T., Regulation of chromatin by histone modifications. *Cell Research* **2011,** *21* (3), 381-395.

10. Arrowsmith, C. H.; Bountra, C.; Fish, P. V.; Lee, K.; Schapira, M., Epigenetic protein families: a new frontier for drug discovery. *Nat Rev Drug Discov* **2012,** *11* (5), 384-400.

11. Cao, J.; Yan, Q., Histone Ubiquitination and Deubiquitination in Transcription, DNA Damage Response, and Cancer. *Frontiers in Oncology* **2012,** *2*, 26.

12. Shiio, Y.; Eisenman, R. N., Histone sumoylation is associated with transcriptional repression. *Proceedings of the National Academy of Sciences* **2003,** *100* (23), 13225-13230.

13. (a) Iyer, A.; Fairlie, D. P.; Brown, L., Lysine acetylation in obesity, diabetes and metabolic disease. *Immunol Cell Biol* **2012,** *90* (1), 39-46; (b) Barnes, P. J.; Adcock, I. M.; Ito, K., Histone acetylation and deacetylation: importance in inflammatory lung diseases. *European Respiratory Journal* **2005,** *25* (3), 552-563.

14. Marks, P. A.; Xu, W. S., Histone Deacetylase Inhibitors: Potential in Cancer Therapy. *Journal of cellular biochemistry* **2009,** *107* (4), 600-608.

15. Picaud, S.; Wells, C.; Felletar, I.; Brotherton, D.; Martin, S.; Savitsky, P.; Diez-Dacal, B.; Philpott, M.; Bountra, C.; Lingard, H.; Fedorov, O.; Müller, S.; Brennan, P. E.; Knapp, S.; Filippakopoulos, P., RVX-208, an inhibitor of BET transcriptional regulators with selectivity for the second bromodomain. *Proceedings of the National Academy of Sciences* **2013,** *110* (49), 19754-19759.

16. Gajer, J. M.; Furdas, S. D.; Grunder, A.; Gothwal, M.; Heinicke, U.; Keller, K.; Colland, F.; Fulda, S.; Pahl, H. L.; Fichtner, I.; Sippl, W.; Jung, M., Histone acetyltransferase inhibitors block neuroblastoma cell growth in vivo. *Oncogenesis* **2015,** *4*, e137.

17. Tamkun, J. W.; Deuring, R.; Scott, M. P.; Kissinger, M.; Pattatucci, A. M.; Kaufman, T. C.; Kennison, J. A., brahma: A regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2SWI2. *Cell* **1992,** *68* (3), 561-572.

18. Dhalluin, C.; Carlson, J. E.; Zeng, L.; He, C.; Aggarwal, A. K.; Zhou, M. M., Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **1999,** *399* (6735), 491-496.

19. Jacobson, R. H.; Ladurner, A. G.; King, D. S.; Tjian, R., Structure and function of a human TAF(II)250 double bromodomain module. *Science* **2000,** *288* (5470), 1422-1425.

20. Hay, D. A.; Fedorov, O.; Martin, S.; Singleton, D. C.; Tallant, C.; Wells, C.; Picaud, S.; Philpott, M.; Monteiro, O. P.; Rogers, C. M.; Conway, S. J.; Rooney, T. P. C.; Tumber, A.; Yapp, C.; Filippakopoulos, P.; Bunnage, M. E.; Müller, S.; Knapp, S.; Schofield, C. J.; Brennan, P. E., Discovery and Optimization of Small-Molecule Ligands for the CBP/p300 Bromodomains. *Journal of the American Chemical Society* **2014,** *136* (26), 9308-9319.

21. Yang, X.-J., MOZ and MORF acetyltransferases: Molecular interaction, animal development and human disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **2015,** *1853* (8), 1818-1826.

22. Filippakopoulos, P.; Picaud, S.; Mangos, M.; Keates, T.; Lambert, J. P.; Barsyte-Lovejoy, D.; Felletar, I.; Volkmer, R.; Muller, S.; Pawson, T.; Gingras, A. C.; Arrowsmith, C. H.; Knapp, S., Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* **2012,** *149* (1), 214-31.

23. Filippakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Felletar, I.; Philpott, M.; Munro, S.; McKeown, M. R.; Wang, Y.; Christie, A. L.; West, N.; Cameron, M. J.; Schwartz, B.; Heightman, T. D.; La Thangue, N.; French, C. A.; Wiest, O.; Kung, A. L.; Knapp, S.; Bradner, J. E., Selective inhibition of BET bromodomains. *Nature* **2010,** *468* (7327), 1067-1073.

24. Wyspianska, B. S.; Bannister, A. J.; Barbieri, I.; Nangalia, J.; Godfrey, A.; Calero-Nieto, F. J.; Robson, S.; Rioja, I.; Li, J.; Wiese, M.; Cannizzaro, E.; Dawson, M. A.; Huntly, B.; Prinjha, R. K.; Green, A.

R.; Gottgens, B.; Kouzarides, T., BET protein inhibition shows efficacy against JAK2V617F-driven neoplasms. *Leukemia* **2014,** *28* (1), 88-97.

25. Nicodeme, E.; Jeffrey, K. L.; Schaefer, U.; Beinke, S.; Dewell, S.; Chung, C. W.; Chandwani, R.; Marazzi, I.; Wilson, P.; Coste, H.; White, J.; Kirilovsky, J.; Rice, C. M.; Lora, J. M.; Prinjha, R. K.; Lee, K.; Tarakhovsky, A., Suppression of inflammation by a synthetic histone mimic. *Nature* **2010,** *468* (7327), 1119-23.

26. Zeng, L.; Zhou, M.-M., Bromodomain: an acetyl-lysine binding domain. *FEBS Letters* **2002,** *513* (1), 124-128.

27. Mirguet, O.; Gosmini, R.; Toum, J.; Clément, C. A.; Barnathan, M.; Brusq, J.-M.; Mordaunt, J. E.; Grimes, R. M.; Crowe, M.; Pineau, O.; Ajakane, M.; Daugan, A.; Jeffrey, P.; Cutler, L.; Haynes, A. C.; Smithers, N. N.; Chung, C.-w.; Bamborough, P.; Uings, I. J.; Lewis, A.; Witherington, J.; Parr, N.; Prinjha, R. K.; Nicodème, E., Discovery of Epigenetic Regulator I-BET762: Lead Optimization to Afford a Clinical Candidate Inhibitor of the BET Bromodomains. *Journal of Medicinal Chemistry* **2013,** *56* (19), 7501-7515.

28. http://www.thesgc.org/chemical-probes/OF-1 (accessed 12/08/2016).

29. Matzuk, Martin M.; McKeown, Michael R.; Filippakopoulos, P.; Li, Q.; Ma, L.; Agno, Julio E.; Lemieux, Madeleine E.; Picaud, S.; Yu, Richard N.; Qi, J.; Knapp, S.; Bradner, James E., Small-Molecule Inhibition of BRDT for Male Contraception. *Cell* **2012,** *150* (4), 673-684.

30. Korb, E.; Herre, M.; Zucker-Scharff, I.; Darnell, R. B.; Allis, C. D., BET protein Brd4 activates transcription in neurons and BET inhibitor Jq1 blocks memory in mice. *Nat Neurosci* **2015,** *advance online publication*.

31. Middeljans, E.; Wan, X.; Jansen, P. W.; Sharma, V.; Stunnenberg, H. G.; Logie, C., SS18 Together with Animal-Specific Factors Defines Human BAF-Type SWI/SNF Complexes. *PLoS ONE* **2012,** *7* (3), e33834.

32. Kadoch, C.; Hargreaves, D. C.; Hodges, C.; Elias, L.; Ho, L.; Ranish, J.; Crabtree, G. R., Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat Genet* **2013,** *45* (6), 592-601.

33. Scotto, L.; Narayan, G.; Nandula, S. V.; Subramaniyam, S.; Kaufmann, A. M.; Wright, J. D.; Pothuri, B.; Mansukhani, M.; Schneider, A.; Arias-Pulido, H.; Murty, V. V., Integrative genomics analysis of chromosome 5p gain in cervical cancer reveals target over-expressed genes, including Drosha. *Molecular Cancer* **2008,** *7*, 58-58.

34. Chiu, Y.-H.; Lee, Jennifer Y.; Cantley, Lewis C., BRD7, a Tumor Suppressor, Interacts with p85α and Regulates PI3K Activity. *Molecular Cell 54* (1), 193-202.

35. (a) Caron, C.; Lestrat, C.; Marsal, S.; Escoffier, E.; Curtet, S.; Virolle, V.; Barbry, P.; Debernardi, A.; Brambilla, C.; Brambilla, E.; Rousseaux, S.; Khochbin, S., Functional characterization

of ATAD2 as a new cancer/testis factor and a predictor of poor prognosis in breast and lung cancers. *Oncogene* **2010,** *29* (37), 5171-81; (b) Zou, J. X.; Guo, L.; Revenko, A. S.; Tepper, C. G.; Gemo, A. T.; Kung, H. J.; Chen, H. W., Androgen-induced coactivator ANCCA mediates specific androgen receptor signaling in prostate cancer. *Cancer research* **2009,** *69* (8), 3339-46.

36. Ciro, M.; Prosperini, E.; Quarto, M.; Grazini, U.; Walfridsson, J.; McBlane, F.; Nucifero, P.; Pacchiana, G.; Capra, M.; Christensen, J.; Helin, K., ATAD2 is a novel cofactor for MYC, overexpressed and amplified in aggressive tumors. *Cancer research* **2009,** *69* (21), 8491-8.

37. Demont, E. H.; Bamborough, P.; Chung, C.-w.; Craggs, P. D.; Fallon, D.; Gordon, L. J.; Grandi, P.; Hobbs, C. I.; Hussain, J.; Jones, E. J.; Le Gall, A.; Michon, A.-M.; Mitchell, D. J.; Prinjha, R. K.; Roberts, A. D.; Sheppard, R. J.; Watson, R. J., 1,3-Dimethyl Benzimidazolones Are Potent, Selective Inhibitors of the BRPF1 Bromodomain. *ACS Medicinal Chemistry Letters* **2014,** *5* (11), 1190-1195.

38. Thompson, K. A.; Wang, B. C.; Argraves, W. S.; Giancotti, F. G.; Ruoslahti, E.; Schranck, D. P., BR140, a Novel Zinc-Finger Protein with Homology to the TAF250 Subunit of TFIID. *Biochemical and Biophysical Research Communications* **1994,** *198* (3), 1143-1152.

39. Tain, Y.-L.; Lee, C.-T.; Huang, L.-T., Long-Term Effects of Maternal Citrulline Supplementation on Renal Transcriptome Prevention of Nitric Oxide Depletion-Related Programmed Hypertension: The Impact of Gene-Nutrient Interactions. *International Journal of Molecular Sciences* **2014,** *15* (12), 23255.

40. Chan-on, W.; Nairismagi, M.-L.; Ong, C. K.; Lim, W. K.; Dima, S.; Pairojkul, C.; Lim, K. H.; McPherson, J. R.; Cutcutache, I.; Heng, H. L.; Ooi, L.; Chung, A.; Chow, P.; Cheow, P. C.; Lee, S. Y.; Choo, S. P.; Tan, I. B. H.; Duda, D.; Nastase, A.; Myint, S. S.; Wong, B. H.; Gan, A.; Rajasegaran, V.; Ng, C. C. Y.; Nagarajan, S.; Jusakul, A.; Zhang, S.; Vohra, P.; Yu, W.; Huang, D.; Sithithaworn, P.; Yongvanit, P.; Wongkham, S.; Khuntikeo, N.; Bhudhisawasdi, V.; Popescu, I.; Rozen, S. G.; Tan, P.; Teh, B. T., Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. *Nat Genet* **2013,** *45* (12), 1474-1478.

41. Tellier, J.; Andrianjaka, A.; Vicente, R.; Thiault, N.; Enault, G.; Garchon, H.-J.; van Meerwijk, J. P. M.; Romagnoli, P., Increased thymic development of regulatory T cells in NOD mice is functionally dissociated from type I diabetes susceptibility. *European Journal of Immunology* **2013,** *43* (5), 1356-1362.

42. Mishima, Y.; Miyagi, S.; Saraya, A.; Negishi, M.; Endoh, M.; Endo, T. A.; Toyoda, T.; Shinga, J.; Katsumoto, T.; Chiba, T.; Yamaguchi, N.; Kitabayashi, I.; Koseki, H.; Iwama, A., The Hbo1-Brd1/Brpf2 complex is responsible for global acetylation of H3K14 and required for fetal liver erythropoiesis. *Blood* **2011,** *118* (9), 2443-2453.

43. Kueh, A. J.; Dixon, M. P.; Voss, A. K.; Thomas, T., HBO1 Is Required for H3K14 Acetylation and Normal Transcriptional Activity during Embryonic Development. *Molecular and Cellular Biology* **2011,** *31* (4), 845-860.

44. Mishima, Y.; Wang, C.; Miyagi, S.; Saraya, A.; Hosokawa, H.; Mochizuki-Kashio, M.; Nakajima-Takagi, Y.; Koide, S.; Negishi, M.; Sashida, G.; Naito, T.; Ishikura, T.; Onodera, A.; Nakayama, T.; Tenen, D. G.; Yamaguchi, N.; Koseki, H.; Taniuchi, I.; Iwama, A., Histone acetylation mediated by Brd1 is crucial for Cd8 gene activation during early thymocyte development. *Nature communications* **2014,** *5*, 5872.

45. Fryland, T.; Elfving, B.; Christensen, J. H.; Mors, O.; Wegener, G.; Borglum, A. D., Electroconvulsive seizures regulates the Brd1 gene in the frontal cortex and hippocampus of the adult rat. *Neuroscience letters* **2012,** *516* (1), 110-3.

46. Poplawski, A.; Hu, K.; Lee, W.; Natesan, S.; Peng, D.; Carlson, S.; Shi, X.; Balaz, S.; Markley, J. L.; Glass, K. C., Molecular Insights into the Recognition of N-Terminal Histone Modifications by the BRPF1 Bromodomain. *Journal of Molecular Biology* **2014,** *426* (8), 1661-1676.

47. Brown, T.; Swansbury, J.; Taj, M. M., Prognosis of patients with t(8;16)(p11;p13) acute myeloid leukemia. *Leukemia & Lymphoma* **2011,** *53* (2), 338-341.

48. Chaikuad, A.; Lang, S.; Brennan, P. E.; Temperini, C.; Fedorov, O.; Hollander, J.; Nachane, R.; Abell, C.; Müller, S.; Siegal, G.; Knapp, S., Structure-Based Identification of Inhibitory Fragments Targeting the p300/CBP-Associated Factor Bromodomain. *Journal of Medicinal Chemistry* **2016,** *59* (4), 1648-1653.

49. Toda, J.; Sakagami, M.; Goan, Y.; Simakata, M.; Saitoh, T.; Horiguchi, Y.; Sano, T., Synthesis of Methoxy-2-quinolones via Pummerer-type Cyclization of N-Aryl-N-methyl-3-(phenylsulfinyl)propionamides. *CHEMICAL & PHARMACEUTICAL BULLETIN* **2000,** *48* (12), 1854-1861.

50. (a) Bunnage, M. E.; Chekler, E. L. P.; Jones, L. H., Target validation using chemical probes. *Nat Chem Biol* **2013,** *9* (4), 195-199; (b) Workman, P.; Collins, I., Probing the Probes: Fitness Factors For Small Molecule Tools. *Chemistry & Biology* **2010,** *17* (6), 561-577.

51. Clark, P. G. K.; Vieira, L. C. C.; Tallant, C.; Fedorov, O.; Singleton, D. C.; Rogers, C. M.; Monteiro, O. P.; Bennett, J. M.; Baronio, R.; Müller, S.; Daniels, D. L.; Méndez, J.; Knapp, S.; Brennan, P. E.; Dixon, D. J., LP99: Discovery and Synthesis of the First Selective BRD7/9 Bromodomain Inhibitor. *Angewandte Chemie International Edition* **2015,** *54* (21), 6217-6221.

52. Romero, F. A.; Taylor, A. M.; Crawford, T. D.; Tsui, V.; Côté, A.; Magnuson, S., Disrupting Acetyl-Lysine Recognition: Progress in the Development of Bromodomain Inhibitors. *Journal of Medicinal Chemistry* **2016,** *59* (4), 1271-1298.

53. Fedorov, O.; Castex, J.; Tallant, C.; Owen, D. R.; Martin, S.; Aldeghi, M.; Monteiro, O.; Filippakopoulos, P.; Picaud, S.; Trzupek, J. D.; Gerstenberger, B. S.; Bountra, C.; Willmann, D.; Wells, C.; Philpott, M.; Rogers, C.; Biggin, P. C.; Brennan, P. E.; Bunnage, M. E.; Schüle, R.; Günther, T.; Knapp, S.; Müller, S., Selective targeting of the BRG/PB1 bromodomains impairs embryonic and trophoblast stem cell maintenance. *Science Advances* **2015,** *1* (10).

54. Vidler, L. R.; Brown, N.; Knapp, S.; Hoelder, S., Druggability Analysis and Structural Classification of Bromodomain Acetyl-lysine Binding Sites. *Journal of Medicinal Chemistry* **2012,** *55* (17), 7346-7359.

55. Chaikuad, A.; Petros, A. M.; Fedorov, O.; Xu, J.; Knapp, S., Structure-based approaches towards identification of fragments for the low-druggability ATAD2 bromodomain. *MedChemComm* **2014,** *5* (12), 1843-1848.

56. Harner, M. J.; Chauder, B. A.; Phan, J.; Fesik, S. W., Fragment-Based Screening of the Bromodomain of ATAD2. *Journal of Medicinal Chemistry* **2014,** *57* (22), 9687-9692.

57. (a) Bamborough, P.; Chung, C.-w.; Furze, R. C.; Grandi, P.; Michon, A.-M.; Sheppard, R. J.; Barnett, H.; Diallo, H.; Dixon, D. P.; Douault, C.; Jones, E. J.; Karamshi, B.; Mitchell, D. J.; Prinjha, R. K.; Rau, C.; Watson, R. J.; Werner, T.; Demont, E. H., Structure-Based Optimization of Naphthyridones into Potent ATAD2 Bromodomain Inhibitors. *Journal of Medicinal Chemistry* **2015,** *58* (15), 6151-6178; (b) Demont, E. H.; Chung, C.-w.; Furze, R. C.; Grandi, P.; Michon, A.-M.; Wellaway, C.; Barrett, N.; Bridges, A. M.; Craggs, P. D.; Diallo, H.; Dixon, D. P.; Douault, C.; Emmons, A. J.; Jones, E. J.; Karamshi, B. V.; Locke, K.; Mitchell, D. J.; Mouzon, B. H.; Prinjha, R. K.; Roberts, A. D.; Sheppard, R. J.; Watson, R. J.; Bamborough, P., Fragment-Based Discovery of Low-Micromolar ATAD2 Bromodomain Inhibitors. *Journal of Medicinal Chemistry* **2015,** *58* (14), 5649-5673.

58. Theodoulou, N. H.; Bamborough, P.; Bannister, A. J.; Becher, I.; Bit, R. A.; Che, K. H.; Chung, C.-w.; Dittmann, A.; Drewes, G.; Drewry, D. H.; Gordon, L.; Grandi, P.; Leveridge, M.; Lindon, M.; Michon, A.-M.; Molnar, J.; Robson, S. C.; Tomkinson, N. C. O.; Kouzarides, T.; Prinjha, R. K.; Humphreys, P. G., Discovery of I-BRD9, a Selective Cell Active Chemical Probe for Bromodomain Containing Protein 9 Inhibition. *Journal of Medicinal Chemistry* **2016,** *59* (4), 1425-1439.

59. Hay, D. A.; Rogers, C. M.; Fedorov, O.; Tallant, C.; Martin, S.; Monteiro, O. P.; Muller, S.; Knapp, S.; Schofield, C. J.; Brennan, P. E., Design and synthesis of potent and selective inhibitors of BRD7 and BRD9 bromodomains. *MedChemComm* **2015,** *6* (7), 1381-1386.

60. Bamborough, P.; Barnett, H. A.; Becher, I.; Bird, M. J.; Chung, C.-w.; Craggs, P. D.; Demont, E. H.; Diallo, H.; Fallon, D. J.; Gordon, L. J.; Grandi, P.; Hobbs, C. I.; Hooper-Greenhill, E.; Jones, E. J.; Law, R. P.; Le Gall, A.; Lugo, D.; Michon, A.-M.; Mitchell, D. J.; Prinjha, R. K.; Sheppard, R. J.; Watson, A. J. B.; Watson, R. J., GSK6853, a Chemical Probe for Inhibition of the BRPF1 Bromodomain. *ACS Medicinal Chemistry Letters* **2016,** *7* (6), 552-557.

61. Bennett, J.; Fedorov, O.; Tallant, C.; Monteiro, O.; Meier, J.; Gamble, V.; Savitsky, P.; Nunez-Alonso, G. A.; Haendler, B.; Rogers, C.; Brennan, P. E.; Müller, S.; Knapp, S., Discovery of a Chemical Tool Inhibitor Targeting the Bromodomains of TRIM24 and BRPF. *Journal of Medicinal Chemistry* **2016,** *59* (4), 1642-1647.

62. Palmer, W. S.; Poncet-Montange, G.; Liu, G.; Petrocchi, A.; Reyna, N.; Subramanian, G.; Theroff, J.; Yau, A.; Kost-Alimova, M.; Bardenhagen, J. P.; Leo, E.; Shepard, H. E.; Tieu, T. N.; Shi, X.; Zhan, Y.; Zhao, S.; Barton, M. C.; Draetta, G.; Toniatti, C.; Jones, P.; Geck Do, M.; Andersen, J. N.,

Structure-Guided Design of IACS-9571, a Selective High-Affinity Dual TRIM24-BRPF1 Bromodomain Inhibitor. *Journal of Medicinal Chemistry* **2016,** *59* (4), 1440-1454.

63. Niesen, F. H.; Berglund, H.; Vedadi, M., The use of differential scanning fluorimetry to detect ligand interactions that promote protein stability. *Nat. Protocols* **2007,** *2* (9), 2212-2221.

64. Fabian, M. A.; Biggs, W. H.; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J.-M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J., A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotech* **2005,** *23* (3), 329-336.

65. http://www.wiredchemist.com/chemistry/data/bond_energies_lengths.html.

66. Ying, B.; Xu, J.; Zhu, X.; Shen, C.; Zhang, P., Catalyst-Controlled Selectivity in the Synthesis of C2- and C3-Sulfonate Esters from Quinoline N-Oxides and Aryl Sulfonyl Chlorides. *ChemCatChem* **2016,** *8* (16), 2604-2608.

67. Nishiwaki, N.; Sakashita, M.; Azuma, M.; Tanaka, C.; Tamura, M.; Asaka, N.; Hori, K.; Tohda, Y.; Ariga, M., Novel functionalization of 1-methyl-2-quinolone; dimerization and denitration of trinitroquinolone. *Tetrahedron* **2002,** *58* (3), 473-478.

68. Hansch, C.; Leo, A.; Taft, R. W., A survey of Hammett substituent constants and resonance and field parameters. *Chemical Reviews* **1991,** *91* (2), 165-195.

69. Jones, L. H.; Summerhill, N. W.; Swain, N. A.; Mills, J. E., Aromatic chloride to nitrile transformation: medicinal and synthetic chemistry. *MedChemComm* **2010,** *1* (5), 309-318.

70. Huggins, M. T.; Barber, P. S.; Florian, D.; Howton, W., Short, Efficient Syntheses of Pyrrole α-Amides. *Synthetic Communications* **2008,** *38* (23), 4226-4239.

71. Fonseca, A. S. C.; Gonçalves, M. S. T.; Costa, S. P. G., Light-induced cleavage of model phenylalanine conjugates based on coumarins and quinolones. *Amino Acids* **2010,** *39* (3), 699-712.

72. van Oeveren, A.; Motamedi, M.; Mani, N. S.; Marschke, K. B.; López, F. J.; Schrader, W. T.; Negro-Vilar, A.; Zhi, L., Discovery of 6-N,N-Bis(2,2,2-trifluoroethyl)amino- 4-trifluoromethylquinolin-2(1H)-one as a Novel Selective Androgen Receptor Modulator#. *Journal of Medicinal Chemistry* **2006,** *49* (21), 6143-6146.

73. Tashima, T.; Murata, H.; Kodama, H., Design and synthesis of novel and highly-active pan-histone deacetylase (pan-HDAC) inhibitors. *Bioorganic & Medicinal Chemistry* **2014,** *22* (14), 3720-3731.

74. Bahrami, K.; Khodaei, M. M.; Soheilizad, M., Direct Conversion of Thiols to Sulfonyl Chlorides and Sulfonamides. *The Journal of Organic Chemistry* **2009,** *74* (24), 9287-9291.

75. Huang, J.; Widlanski, T. S., Facile synthesis of sulfonyl chlorides. *Tetrahedron Letters* **1992,** *33* (19), 2657-2660.

76. DeBergh, J. R.; Niljianskul, N.; Buchwald, S. L., Synthesis of Aryl Sulfonamides via Palladium-Catalyzed Chlorosulfonylation of Arylboronic Acids. *Journal of the American Chemical Society* **2013,** *135* (29), 10638-10641.

77. Meerwein, H.; Dittmar, G.; Göllner, R.; Hafner, K.; Mensch, F.; Steinfort, O., Untersuchungen über aromatische Diazoverbindungen, II. Verfahren zur Herstellung Aromatischer Sulfonsäurechloride, Eine Neue Modifikation der Sandmeyerschen Reaktion. *Chemische Berichte* **1957,** *90* (6), 841-852.

78. Hogan, P. J.; Cox, B. G., Aqueous Process Chemistry: The Preparation of Aryl Sulfonyl Chlorides. *Organic Process Research & Development* **2009,** *13* (5), 875-879.

79. Majumdar, K. C.; Chattopadhyay, B.; Samanta, S., A Short Route to the Synthesis of Pyrrolocoumarin and Pyrroloquinolone Derivatives by Sonogashira Cross-Coupling and Gold-Catalyzed Cycloisomerization of Acetylenic Amines. *Synthesis* **2009,** *2009* (02), 311-317.

80. Karramkam, M.; Dollé, F.; Valette, H.; Besret, L.; Bramoullé, Y.; Hinnen, F.; Vaufrey, F.; Franklin, C.; Bourg, S.; Coulon, C.; Ottaviani, M.; Delaforge, M.; Loc'h, C.; Bottlaender, M.; Crouzel, C., Synthesis of a fluorine-18-labelled derivative of 6-nitroquipazine, as a radioligand for the In vivo serotonin transporter imaging with PET. *Bioorganic & Medicinal Chemistry* **2002,** *10* (8), 2611-2623.

81. Turner, J. A., A general approach to the synthesis of 1,6-, 1,7-, and 1,8-naphthyridines. *The Journal of Organic Chemistry* **1990,** *55* (15), 4744-4750.

82. Ooi, T.; Takahashi, M.; Yamada, M.; Tayama, E.; Omoto, K.; Maruoka, K., (2,7-Disubstituted-1,8-biphenylenedioxy)bis(dimethylaluminum) as Bidentate Organoaluminum Lewis Acids: Elucidation and Synthetic Utility of the Double Electrophilic Activation Phenomenon. *Journal of the American Chemical Society* **2004,** *126* (4), 1150-1160.

83. Bertani, B.; Borriello, M.; Bozzoli, A.; Bromidge, S. M.; Granci, E.; Leslie, C.; Serafinowska, H.; Stasi, L.; Vong, A.; Zucchelli, V., Benzoxazinone derivatives, preparation thereof and uses in the treatment of cns and other disorders. Google Patents: 2004.

84. Arora, N.; Chen, S.; Hermann, J. C.; Kuglstatter, A.; Labadie, S. S.; Lin, C. J. J.; Lucas, M. C.; Moore, A. G.; Papp, E.; Talamas, F. X., PYRAZOLO[1,5a]PYRIMIDINE DERIVATIVES AS IRAK4 MODULATORS. Google Patents: 2012.

85. Zhang, Z.; Tillekeratne, L. M. V.; Hudson, R. A., Synthesis of Isomeric Analogues of Coenzyme Pyrroloquinoline Quinone (PQQ). *Synthesis* **1996,** *1996* (03), 377-382.

86. Crisenza, G. E. M.; Sokolova, O. O.; Bower, J. F., Branch-Selective Alkene Hydroarylation by Cooperative Destabilization: Iridium-Catalyzed ortho-Alkylation of Acetanilides. *Angewandte Chemie International Edition* **2015,** *54* (49), 14866-14870.

87. Fernandez, M.; de la Cuesta, E.; Avendano, C., Synthesis of 5-Methoxy-2 (1H)-quinolinone. *ChemInform* **1995,** *26* (20).

88. Xu, H.; Wolf, C., Efficient copper-catalyzed coupling of aryl chlorides, bromides and iodides with aqueous ammonia. *Chemical Communications* **2009,** (21), 3035-3037.

89. Ge, H.-X.; Wang, L.-C.; Jiang, Z.-Z.; Ni, S.-L., Synthesis and Bioactivity of Aripiprazole Derivatives. *Arzneimittelforschung* **2006,** *56* (10), 673-677.

90. Hu, Q.; Yin, L.; Hartmann, R. W., Selective Dual Inhibitors of CYP19 and CYP11B2: Targeting Cardiovascular Diseases Hiding in the Shadow of Breast Cancer. *Journal of Medicinal Chemistry* **2012,** *55* (16), 7080-7089.

91. Popp, F. D.; McEwen, W. E., Polyphosphoric Acids As A Reagent In Organic Chemistry. *Chemical Reviews* **1958,** *58* (2), 321-401.

92. Fish, P. V.; Cook, A. S.; Phillips, C.; BENT, A. F.; Mills, J. E. J.; Sciammetta, N., Novel heterocyclic compounds as bromodomain inhibitors. Google Patents: 2013.

93. Wu, J.; Shin, J.; Williams, C. M. M.; Geoghegan, K. F.; Wright, S. W.; Limburg, D. C.; Sahasrabudhe, P.; Bonin, P. D.; Lefker, B. A.; Ramsey, S. J., Design and chemoproteomic functional characterization of a chemical probe targeted to bromodomains of BET family proteins. *MedChemComm* **2014,** *5* (12), 1871-1878.

94. Meth-Cohn, O.; Narine, B.; Tarnowski, B., A versatile new synthesis of quinolines and related fused pyridines, Part 5. The synthesis of 2-chloroquinoline-3-carbaldehydes. *Journal of the Chemical Society, Perkin Transactions 1* **1981,** (0), 1520-1530.

95. Marsais, F.; Godard, A.; Queguiner, G., Directed ortho-lithiation of chloroquinolines. Application to synthesis of 2,3-disubstituted quinolines. *Journal of Heterocyclic Chemistry* **1989,** *26* (6), 1589-1594.

96. Nishiwaki, N.; Tanaka, C.; Asahara, M.; Asaka, N.; Tohda, Y.; Ariga, M., A nitro group distorting 2-quinolone skeleton. *Heterocycles* **1999,** *51* (3), 567-574.

97. Ulysse, L. G.; Yang, Q.; McLaws, M. D.; Keefe, D. K.; Guzzo, P. R.; Haney, B. P., Process Development and Pilot-Scale Synthesis of New Cyclization Conditions of Substituted Phenylacetamides to Tetrahydroisoquinoline-2-ones Using Eaton's Reagent. *Organic Process Research & Development* **2010,** *14* (1), 225-228.

98. Grongsaard, P.; Bulger, P. G.; Wallace, D. J.; Tan, L.; Chen, Q.; Dolman, S. J.; Nyrop, J.; Hoerrner, R. S.; Weisel, M.; Arredondo, J.; Itoh, T.; Xie, C.; Wen, X.; Zhao, D.; Muzzio, D. J.; Bassan, E.

M.; Shultz, C. S., Convergent, Kilogram Scale Synthesis of an Akt Kinase Inhibitor. *Organic Process Research & Development* **2012,** *16* (5), 1069-1081.

99. Kompella, A.; Gampa, V. K.; GANGANAMONI, S.; SIRIGIREDDY, B. R.; Adibhatla, K. S. B. R.; Nannapaneni, V. C., 1h-1,8- naphthyridin-2ones as anti proliferative compounds. Google Patents: 2015.

100. Belavagi, N.; Rajagopalan, S.; Ramachandra, M.; Sengupta, S., 2, 4 -diaminopyrimidine derivatives as protein kinase inhibitors. Google Patents: 2012.

101. Kusakabe, K.-i.; Ide, N.; Daigo, Y.; Tachibana, Y.; Itoh, T.; Yamamoto, T.; Hashizume, H.; Hato, Y.; Higashino, K.; Okano, Y.; Sato, Y.; Inoue, M.; Iguchi, M.; Kanazawa, T.; Ishioka, Y.; Dohi, K.; Kido, Y.; Sakamoto, S.; Yasuo, K.; Maeda, M.; Higaki, M.; Ueda, K.; Yoshizawa, H.; Baba, Y.; Shiota, T.; Murai, H.; Nakamura, Y., Indazole-Based Potent and Cell-Active Mps1 Kinase Inhibitors: Rational Design from Pan-Kinase Inhibitor Anthrapyrazolone (SP600125). *Journal of Medicinal Chemistry* **2013,** *56* (11), 4343-4356.

102. Bala, K.; Leblanc, C.; Sandham, D. A.; Turner, K. L.; Watson, S. J.; Brown, L. N.; Cox, B., Pyrrolopyridine derivatives and their use as crth2 antagonists. Google Patents: 2005.

103. Wilkinson, G. R.; Shand, D. G., Commentary: a physiological approach to hepatic drug clearance. *Clinical pharmacology and therapeutics* **1975,** *18* (4), 377-90.

104. Huang, S. M.; Temple, R.; Throckmorton, D. C.; Lesko, L. J., Drug Interaction Studies: Study Design, Data Analysis, and Implications for Dosing and Labeling. *Clinical Pharmacology & Therapeutics* **2007,** *81* (2), 298-304.

105. Y, M., Epigenetics and COPD. 2015.

106. Winter, G. E.; Buckley, D. L.; Paulk, J.; Roberts, J. M.; Souza, A.; Dhe-Paganon, S.; Bradner, J. E., Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* **2015**.

107. Manikandan, R.; Jeganmohan, M., Ruthenium-Catalyzed Cyclization of Anilides with Substituted Propiolates or Acrylates: An Efficient Route to 2-Quinolinones. *Organic Letters* **2014,** *16* (13), 3568-3571.

108. Wu, Y.-L.; Chuang, C.-P.; Lin, P.-Y., Free Radical Cyclization Reactions of Alkylsulfonyl and Alkylthio Substituted Aromatic Amide Derivatives. *Tetrahedron* **2000,** *56* (34), 6209-6217.

109. Gama, F. H. S.; de Souza, R. O. M. A.; Garden, S. J., An efficient green protocol for the preparation of acetoacetamides and application of the methodology to a one-pot synthesis of Biginelli dihydropyrimidines. Expansion of dihydropyrimidine topological chemical space. *RSC Advances* **2015,** *5* (87), 70915-70928.

110. Ferguson, J.; Zeng, F.; Alwis, N.; Alper, H., Synthesis of 2(1H)-Quinolinones via Pd-Catalyzed Oxidative Cyclocarbonylation of 2-Vinylanilines. *Organic Letters* **2013,** *15* (8), 1998-2001.

111. Fish, P. V.; IGOE, N.; BAYLE, E. D., Quinolones as inhibitors of class iv bromodomain proteins. Google Patents: 2016.

112. Du, D. Y.; Procter, M. J.; Fyfe, M. C. T.; Shah, V. K.; Williams, G. M.; Schofield, K. L., Urea and thiourea derivatives. Google Patents: 2004.

113. Suh, Y.-G.; Lee, Y.-S.; Min, K.-H.; Park, O.-H.; Kim, J.-K.; Seung, H.-S.; Seo, S.-Y.; Lee, B.-Y.; Nam, Y.-H.; Lee, K.-O.; Kim, H.-D.; Park, H.-G.; Lee, J.; Oh, U.; Lim, J.-O.; Kang, S.-U.; Kil, M.-J.; Koo, J.-y.; Shin, S. S.; Joo, Y.-H.; Kim, J. K.; Jeong, Y.-S.; Kim, S.-Y.; Park, Y.-H., Novel Potent Antagonists of Transient Receptor Potential Channel, Vanilloid Subfamily Member 1: Structure−Activity Relationship of 1,3-Diarylalkyl Thioureas Possessing New Vanilloid Equivalents. *Journal of Medicinal Chemistry* **2005,** *48* (18), 5823-5836.

114. Choi-Sledeski, Y. M.; McGarry, D. G.; Green, D. M.; Mason, H. J.; Becker, M. R.; Davis, R. S.; Ewing, W. R.; Dankulich, W. P.; Manetta, V. E.; Morris, R. L.; Spada, A. P.; Cheney, D. L.; Brown, K. D.; Colussi, D. J.; Chu, V.; Heran, C. L.; Morgan, S. R.; Bentley, R. G.; Leadley, R. J.; Maignan, S.; Guilloteau, J.-P.; Dunwiddie, C. T.; Pauls, H. W., Sulfonamidopyrrolidinone Factor Xa Inhibitors: Potency and Selectivity Enhancements via P-1 and P-4 Optimization. *Journal of Medicinal Chemistry* **1999,** *42* (18), 3572-3587.

115. Venkov, A. P.; Statkova-Abeghe, S. M., Synthesis of 3,4-dihydroisoquinolines, 2-alkyl(Acyl)-1(2H)-3,4-dihydroisoquinolinones, 2-alkyl-1(2H)-isoquinolinones and 1-alkyl-2(2H)-quinolinones by oxidation with potassium permanganate. *Tetrahedron* **1996,** *52* (4), 1451-1460.

116. Robl, J. A.; Li, J.; Kennedy, L. J.; Walker, S. J.; Wang, H.; Washburn, W. N.; Ahmad, S.; Ngu, K., Novel bicyclic nitrogen containing heteroaryl tgr5 receptor modulators. Google Patents: 2012.

117. Otsubo, N.; Okazaki, S.; Tsukumo, Y.; Iida, K.; Nakoji, M., Pyrimido-diazepinone compound. Google Patents: 2014.

118. Lee, H. G.; Milner, P. J.; Buchwald, S. L., Pd-Catalyzed Nucleophilic Fluorination of Aryl Bromides. *Journal of the American Chemical Society* **2014,** *136* (10), 3792-3795.

119. El-Damasy, A. K.; Seo, S. H.; Cho, N.-C.; Kang, S. B.; Pae, A. N.; Kim, K.-S.; Keum, G., Design, synthesis, in-vitro antiproliferative activity and kinase profile of new picolinamide based 2-amido and ureido quinoline derivatives. *European Journal of Medicinal Chemistry* **2015,** *101*, 754-768.

120. Wang, T.-C.; Chen, Y.-L.; Lee, K.-H.; Tzeng, C.-C., Lewis Acid Catalyzed Reaction of Cinnamanilides: Competition of Intramolecular and Intermolecular Friedel-Crafts Reaction. *Synthesis* **1997,** *1997* (01), 87-90.

121. Chen, Y.; Turlik, A.; Newhouse, T. R., Amide α,β-Dehydrogenation Using Allyl-Palladium Catalysis and a Hindered Monodentate Anilide. *Journal of the American Chemical Society* **2016,** *138* (4), 1166-1169.

122. Bodill, T.; Conibear, A. C.; Mutorwa, M. K. M.; Goble, J. L.; Blatch, G. L.; Lobb, K. A.; Klein, R.; Kaye, P. T., Exploring DOXP-reductoisomerase binding limits using phosphonated N-aryl and N-heteroarylcarboxamides as DXR inhibitors. *Bioorganic & Medicinal Chemistry* **2013,** *21* (14), 4332-4341.

123. Krauss, J.; Knorr, V.; Manhardt, V.; Scheffels, S.; Bracher, F., Synthesis of Platensimycin Analogues and Their Antibiotic Potency. *Archiv der Pharmazie* **2008,** *341* (6), 386-392.

124. Wu, J.; Xiang, S.; Zeng, J.; Leow, M.; Liu, X.-W., Practical Route to 2-Quinolinones via a Pd-Catalyzed C–H Bond Activation/C–C Bond Formation/Cyclization Cascade Reaction. *Organic Letters* **2015,** *17* (2), 222-225.

125. Meyers, A. I.; Temple, D. L.; Nolen, R. L.; Mihelich, E. D., Oxazolines. IX. Synthesis of homologated acetic acids and esters. *The Journal of Organic Chemistry* **1974,** *39* (18), 2778-2783.

126. Sabol, M. R.; Owen, J. M.; Erickson, W. R., A Novel and Efficient Synthesis of 2,3-Dichloroquinoline. *Synthetic Communications* **2000,** *30* (3), 427-432.

127. Verner, E.; Katz, B. A.; Spencer, J. R.; Allen, D.; Hataye, J.; Hruzewicz, W.; Hui, H. C.; Kolesnikov, A.; Li, Y.; Luong, C.; Martelli, A.; Radika, K.; Rai, R.; She, M.; Shrader, W.; Sprengeler, P. A.; Trapp, S.; Wang, J.; Young, W. B.; Mackman, R. L., Development of Serine Protease Inhibitors Displaying a Multicentered Short (<2.3 Å) Hydrogen Bond Binding Mode: Inhibitors of Urokinase-Type Plasminogen Activator and Factor Xa. *Journal of Medicinal Chemistry* **2001,** *44* (17), 2753-2771.

128. Butera, J. A.; Antane, M. M.; Antane, S. A.; Argentieri, T. M.; Freeden, C.; Graceffa, R. F.; Hirth, B. H.; Jenkins, D.; Lennox, J. R.; Matelan, E.; Norton, N. W.; Quagliato, D.; Sheldon, J. H.; Spinelli, W.; Warga, D.; Wojdan, A.; Woods, M., Design and SAR of Novel Potassium Channel Openers Targeted for Urge Urinary Incontinence. 1. N-Cyanoguanidine Bioisosteres Possessing in Vivo Bladder Selectivity. *Journal of Medicinal Chemistry* **2000,** *43* (6), 1187-1202.

129. Diab, S.; Teo, T.; Kumarasiri, M.; Li, P.; Yu, M.; Lam, F.; Basnet, S. K. C.; Sykes, M. J.; Albrecht, H.; Milne, R.; Wang, S., Discovery of 5-(2-(Phenylamino)pyrimidin-4-yl)thiazol-2(3H)-one Derivatives as Potent Mnk2 Inhibitors: Synthesis, SAR Analysis and Biological Evaluation. *ChemMedChem* **2014,** *9* (5), 962-972.

130. Aggarwal, V. K.; Belfield, A. J., Catalytic Asymmetric Nazarov Reactions Promoted by Chiral Lewis Acid Complexes. *Organic Letters* **2003,** *5* (26), 5075-5078.

131. Lin, S.-Y.; Yeh, T.-K.; Kuo, C.-C.; Song, J.-S.; Cheng, M.-F.; Liao, F.-Y.; Chao, M.-W.; Huang, H.-L.; Chen, Y.-L.; Yang, C.-Y.; Wu, M.-H.; Hsieh, C.-L.; Hsiao, W.; Peng, Y.-H.; Wu, J.-S.; Lin, L.-M.; Sun, M.; Chao, Y.-S.; Shih, C.; Wu, S.-Y.; Pan, S.-L.; Hung, M.-S.; Ueng, S.-H., Phenyl Benzenesulfonylhydrazides Exhibit Selective Indoleamine 2,3-Dioxygenase Inhibition with Potent in Vivo Pharmacodynamic Activity and Antitumor Efficacy. *Journal of Medicinal Chemistry* **2016,** *59* (1), 419-430.

132. Mofford, D. M.; Reddy, G. R.; Miller, S. C., Aminoluciferins Extend Firefly Luciferase Bioluminescence into the Near-Infrared and Can Be Preferred Substrates over d-Luciferin. *Journal of the American Chemical Society* **2014,** *136* (38), 13277-13282.

133. Shahin, M. I.; Abou El Ella, D. A.; Ismail, N. S. M.; Abouzid, K. A. M., Design, synthesis and biological evaluation of type-II VEGFR-2 inhibitors based on quinoxaline scaffold. *Bioorganic Chemistry* **2014,** *56*, 16-26.

134. Li, Z.-S.; Wang, W.-X.; Yang, J.-D.; Wu, Y.-W.; Zhang, W., Photoinduced and N-Bromosuccinimide-Mediated Cyclization of 2-Azido-N-phenylacetamides. *Organic Letters* **2013,** *15* (15), 3820-3823.

135. Rewcastle, G. W.; Denny, W. A.; Winters, R. T.; Colbry, N. L.; Showalter, H. D. H., Synthesis of 6-substituted pyrido[3,4-d]pyrimidin-4(3H)-ones via directed lithiation of 2-substituted 5-aminopyridine derivatives. *Journal of the Chemical Society, Perkin Transactions 1* **1996,** (18), 2221-2226.

136. Leivers, A. L.; Tallant, M.; Shotwell, J. B.; Dickerson, S.; Leivers, M. R.; McDonald, O. B.; Gobel, J.; Creech, K. L.; Strum, S. L.; Mathis, A.; Rogers, S.; Moore, C. B.; Botyanszki, J., Discovery of Selective Small Molecule Type III Phosphatidylinositol 4-Kinase Alpha (PI4KIIIα) Inhibitors as Anti Hepatitis C (HCV) Agents. *Journal of Medicinal Chemistry* **2014,** *57* (5), 2091-2106.

137. Murtiashaw, C. W.; Breitenbach, R.; Goldstein, S. W.; Pezzullo, S. L.; Quallich, G. J.; Sarges, R., 2,3-Pyridine annulation. The enantioselective synthesis of an aldose reductase inhibitor. *The Journal of Organic Chemistry* **1992,** *57* (6), 1930-1933.

138. Hibi, S.; Ueno, K.; Nagato, S.; Kawano, K.; Ito, K.; Norimine, Y.; Takenaka, O.; Hanada, T.; Yonaga, M., Discovery of 2-(2-Oxo-1-phenyl-5-pyridin-2-yl-1,2-dihydropyridin-3-yl)benzonitrile (Perampanel): A Novel, Noncompetitive α-Amino-3-hydroxy-5-methyl-4-isoxazolepropanoic Acid (AMPA) Receptor Antagonist. *Journal of Medicinal Chemistry* **2012,** *55* (23), 10584-10600.

139. Zhou, J.; Li, B.; Hu, F.; Shi, B.-F., Rhodium(III)-Catalyzed Oxidative Olefination of Pyridines and Quinolines: Multigram-Scale Synthesis of Naphthyridinones. *Organic Letters* **2013,** *15* (13), 3460-3463.

140. Ryu, K. E.; Kim, B. R.; Sung, G. H.; Yoon, H. J.; Yoon, Y.-J., Facile Synthesis of Benzo[d]azol-2(3H)-ones Using 2-Phenoxycarbonyl-4,5-dichloropyridazin-3(2H)-one as Green CO Source. *Synlett* **2015,** *26* (14), 1985-1990.

141. Conconi, M. T.; Marzaro, G.; Urbani, L.; Zanusso, I.; Di Liddo, R.; Castagliuolo, I.; Brun, P.; Tonus, F.; Ferrarese, A.; Guiotto, A.; Chilin, A., Quinazoline-based multi-tyrosine kinase inhibitors: Synthesis, modeling, antitumor and antiangiogenic properties. *European Journal of Medicinal Chemistry* **2013,** *67*, 373-383.